

# **FAT METABOLISM IN SEVERELY OBESE ADULTS**

by

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Key metabolic impairments in obesity are reduced skeletal muscle and systemic fatty acid oxidation (FAO). Few clinical trials in severely obese adults examined how the BMI classification relates to FAO and how the addition of exercise to weight loss interventions impacts FAO. **PURPOSE:** To examine how BMI levels that include severely obese relate to FAO. To examine how weight loss interventions with or without exercise in severely obese adults impact FAO. **METHODS:** Adults (BMI:20-52 kg/m<sup>2</sup>) were recruited to examine skeletal muscle FAO with histochemical analysis of muscle biopsies and systemic FAO at rest and during submaximal exercise with indirect calorimetry. Changes in skeletal muscle and systemic FAO of severely obese adults randomized to one of the following weight loss interventions were examined: surgery (S), surgery plus exercise (SE) and diet plus exercise (DE). **RESULTS:** BMI was not significantly related to skeletal muscle or systemic FAO at rest. During exercise at an absolute workload, BMI only had a significant moderate, positive association with systemic absolute FAO (mg/min) in the univariate analysis ( $r=0.46, p<0.01$ ). During exercise at the relative workload, BMI had a moderate, negative association with respiratory exchange ratio (RER;  $r=0.54, p<0.01$ ) and moderate, positive associations with absolute FAO ( $r=0.45, p<0.01$ ) and proportion of energy derived from fat (EF;  $r=0.55, p<0.01$ ). When controlling for confounders (age, gender, race, cardiovascular fitness and physical activity), BMI only maintained significance with RER, explaining 21% of the variance ( $p<0.01$ ). Upon completion

of weight loss interventions, change in skeletal muscle or systemic FAO at rest was not significantly different across intervention groups. DE had greater positive changes in RER and EF during submaximal exercise bouts compared to the other groups ( $p < 0.05$ ). SE had significantly greater positive changes compared to S only with FAO normalized to fat free mass during the relative submaximal workload ( $p < 0.01$ ). **CONCLUSIONS:** BMI does not explain the variance in FAO at rest, but is associated with higher FAO during submaximal exercise bouts. Inclusion of exercise to weight loss induced by caloric restriction results in more favorable changes in FAO during submaximal exercise compared to surgery induced weight loss with or without exercise.

## TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS .....</b>	<b>XIV</b>
<b>1.0 INTRODUCTION .....</b>	<b>1</b>
<b>1.1 PURPOSE .....</b>	<b>3</b>
<b>1.2 AIMS AND HYPOTHESES .....</b>	<b>4</b>
<b>1.3 SIGNIFICANCE.....</b>	<b>6</b>
<b>2.0 REVIEW OF LITERATURE .....</b>	<b>8</b>
<b>2.1 OBESITY EPIDEMIC .....</b>	<b>8</b>
<b>2.2 FATTY ACID OXIDATION.....</b>	<b>9</b>
<b>2.2.1 Impaired Resting Fatty Acid Oxidation and Obesity .....</b>	<b>10</b>
<b>2.2.2 Fatty Acid Oxidation in Obesity During Exercise .....</b>	<b>13</b>
<b>2.3 WEIGHT LOSS INTERVENTIONS FOR THE OBESE POPULATION .....</b>	<b>14</b>
<b>2.3.1 Weight Loss and Fat Metabolism in Obesity.....</b>	<b>16</b>
<b>2.3.2 Exercise Intervention and Fat Metabolism in Lean and Obese Adults ....</b>	<b>17</b>
<b>2.3.3 Exercise in Combination with Weight Loss in Obese Adults.....</b>	<b>20</b>
<b>3.0 METHODS .....</b>	<b>22</b>
<b>3.1 SUBJECTS AND SCREENING .....</b>	<b>22</b>
<b>3.2 EXPERIMENTAL PROCEDURES.....</b>	<b>24</b>
<b>3.2.1 Anthropometrics and Body Composition .....</b>	<b>25</b>

3.2.2	Resting Fatty Acid Oxidation.....	26
3.2.3	Physical Activity Assessment .....	27
3.2.4	Peak Aerobic Capacity Test .....	28
3.2.5	Submaximal Exercise Tests.....	29
3.2.6	Muscle Biopsy .....	30
3.3	RANDOMIZATION AND GROUPING FOR INTERVENTION STUDY USED TO EXAMINE AIMS 3 AND 4 .....	32
3.3.1	Diet Only Intervention.....	33
3.3.2	Diet and Exercise Intervention .....	33
3.3.3	Bariatric Surgery and Health Education Intervention .....	34
3.3.4	Bariatric Surgery and Exercise Intervention .....	34
3.4	DATA ANALYSIS.....	35
3.4.1	Statistical Analysis for Cross Sectional Study (Aims 1 and 2).....	35
3.4.2	Statistical Analysis for Longitudinal Intervention Study (Aims 3 and 4). .....	37
3.4.3	Power Analysis .....	38
4.0	RESULTS .....	40
4.1	PARTICIPANTS OF CROSS SECTIONAL STUDY .....	40
4.2	MARKERS OF SKELETAL MUSCLE OXIDATIVE CAPACITY AND INTRAMYOCELLULAR LIPID CONTENT ACROSS BMI SPECTRUM.....	42
4.3	SYSTEMIC FATTY ACID OXIDATION AT REST AND ACROSS BMI SPECTRUM.....	43
4.4	METABOLIC RESPONSES DURING ABSOLUTE WORKLOAD OF SUBMAXIMAL INTENSITY.....	44

<b>4.5</b>	<b>SYSTEMIC FATTY ACID OXIDATION ACROSS BMI SPECTRUM DURING ABSOLUTE WORKLOAD OF SUBMAXIMAL INTENSITY .....</b>	<b>45</b>
<b>4.6</b>	<b>SYSTEMATIC FATTY ACID OXIDATION ACROSS BMI SPECTRUM DURING RELATIVE SUBMAXIMAL EXERCISE BOUT .....</b>	<b>46</b>
<b>4.7</b>	<b>RELATIONSHIP BETWEEN SKELETAL MUSCLE AND SYSTEMIC FATTY ACID OXIDATION.....</b>	<b>52</b>
<b>4.8</b>	<b>SUBJECT CHARACTERISTICS OF SEVERELY OBESE ADULTS IN INTERVENTION STUDY .....</b>	<b>55</b>
<b>4.9</b>	<b>DIFFERENCES IN SKELETAL MUSCLE FATTY ACID OXIDATION CAPACITY AND INTRAMYOCYELLULAR LIPID CONTENT ACROSS INTERVENTION GROUPS .....</b>	<b>57</b>
<b>4.10</b>	<b>CHANGES IN SYSTEMIC FATTY ACID OXIDATION AT REST ACROSS INTERVENTION GROUPS.....</b>	<b>59</b>
<b>4.11</b>	<b>CHANGES IN SYSTEMIC FATTY ACID OXIDATION AT ABSOLUTE SUBMAXIMAL WORKLOAD ACROSS INTERVENTION GROUPS.....</b>	<b>61</b>
<b>4.12</b>	<b>CHANGES IN SYSTEMIC FATTY ACID OXIDATION AT RELATIVE WORKLOAD ACROSS INTERVENTION GROUPS .....</b>	<b>63</b>
<b>4.13</b>	<b>RELATIONSHIPS BETWEEN CHANGES IN MARKERS OF SKELETAL MUSCLE OXIDATIVE CAPACITY AND INTRAMYOCYELLULAR CONTENT AND CHANGES IN SYSTEMIC FATTY ACID OXIDATION .....</b>	<b>66</b>
<b>4.14</b>	<b>RELATIONSHIPS BETWEEN PHYSICAL ACTIVITY ON SKELETAL MUSCLE AND CHANGES IN SYSTEMIC FATTY ACID OXIDATION.....</b>	<b>66</b>
<b>5.0</b>	<b>DISCUSSION .....</b>	<b>67</b>



<b>5.1 MARKERS OF SKELETAL MUSCLE OXIDATIVE CAPACITY AND INTRAMYOCELLULAR LIPID CONTENT ACROSS BMI SPECTRUM.....</b>	<b>67</b>
<b>5.2 RELATIONSHIP BETWEEN SKELETAL MUSCLE AND SYSTEMIC FATTY ACID OXIDATION.....</b>	<b>73</b>
<b>5.3 CHANGES IN FATTY ACID OXIDATION ACROSS WEIGHT LOSS INTERVENTION GROUPS .....</b>	<b>74</b>
<b>BIBLIOGRAPHY .....</b>	<b>81</b>

## LIST OF TABLES

Table 1. Variables and units of skeletal muscle markers of oxidative capacity, intramyocellular lipid (IMCL) and systemic fatty acid oxidation (FAO). .....	36
Table 2. Characteristics by BMI classification and for the total sample of participants in the cross sectional study. Presented in medians and quartiles unless otherwise noted. ....	41
Table 3. Markers of skeletal muscle fatty acid oxidative (FAO) capacity and intramyocellular lipid (IMCL) content by body mass index (BMI) classification and for total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between markers of skeletal muscle FAO capacity and IMCL content with predictor variables in unadjusted and adjusted models are also presented. ....	42
Table 4. Systemic fatty acid oxidation (FAO) at rest by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of systemic FAO with predictor variables in unadjusted and adjusted models are also presented. ....	43
Table 5. Metabolic responses during the absolute submaximal workload (25 W) by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between these variables and BMI are also presented. ....	44

Table 6. Systemic fatty acid oxidation (FAO) measured during the absolute submaximal workload by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of FAO with predictor variables in unadjusted and adjusted models are also presented.....	45
Table 7. Systemic fatty acid oxidation (FAO) measured during the absolute submaximal workload by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of FAO with predictor variables in unadjusted and adjusted models are also presented.....	47
Table 8. Systemic fatty acid oxidation (FAO) measured during the relative submaximal workload by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of FAO with predictor variables in unadjusted and adjusted models are also presented.....	48
Table 9. Multivariable models testing the relationships between BMI and absolute fatty acid oxidation (mg/min) during a submaximal relative workload, controlling for potential confounders (N=34).....	52
Table 10. Correlations between systemic fatty acid oxidation (FAO) at rest and skeletal muscle oxidative capacity and intramyocellular (IMCL) content. Presented correlation coefficients/p values. ....	53
Table 11. Correlations between systemic fatty acid oxidation (FAO) during the relative submaximal exercise bout and skeletal muscle oxidative capacity and IMCL content. Presented correlation coefficients/p values. ....	54
Table 12. Baseline subject characteristics by intervention group (n=24) presented in medians and quartiles unless otherwise stated.....	56

Table 13. Changes in body weight and physical activity by intervention group (n=24) from baseline to 6 months. Presented in medians and quartiles. ....	57
Table 14. Differences in skeletal muscle oxidative capacity and intramyocellular (IMCL) content across intervention groups. Presented in medians and quartiles. ....	58
Table 15. Changes in systemic fatty acid oxidation (FAO) at rest across intervention groups. Presented in medians and quartiles. ....	60
Table 16. Changes in systemic fatty acid oxidation (FAO) measured during the absolute submaximal workload across intervention groups. Presented in medians and quartiles. ....	62
Table 17. Changes in FAO variables during relative submaximal workload across intervention groups. Presented in medians and quartiles. ....	65

## LIST OF FIGURES

Figure 1. Absolute fatty acid oxidation (FAO) during the absolute submaximal workload across by BMI ( $r=0.46$ , $p<0.01$ ). .....	46
Figure 2. Respiratory exchange ratio (RER) during the relative submaximal workload across by BMI ( $r=-0.54$ , $p<0.01$ ). .....	49
Figure 3. Absolute fatty acid oxidation (FAO) during the relative submaximal workload across by BMI ( $r=0.45$ , $p<0.01$ ). .....	50
Figure 4. Energy derived from fat (EF) during the relative submaximal workload across by BMI ( $r=0.55$ , $p<0.01$ ). .....	51

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## **1.0 INTRODUCTION**

The percent of adults classified as obese more than doubled from 15% in 1980 to 32.2% in 2004 (Ogden, 2006), and shifted toward a greater prevalence of extreme obesity (Freedman, 2002). The highest classification of BMI is associated with the greatest risk of developing chronic health conditions (Must, 1999) and also the greatest risk of mortality (Calle, 1999). The resulting epidemic related to obesity heightens the need for the clinical investigation of the prevention and treatment of severe obesity.

A lower systemic rate of fatty acid oxidation (FAO) at rest is a risk factor for weight gain (Zurlo, 1990). In comparison to lean adults, obese adults present with a decrement in the capacity of skeletal muscle FAO when examined with the limb balance technique (Kelley, 1999). Fatty acids that are not oxidized accumulate within non-adipose tissues and organs such as skeletal muscle. Intramuscular lipid (IMCL) content as measured by computed tomography scans (Goodpaster, 1997; Simoneau, 1995) and percutaneous muscle biopsies (Levin, 2001; Pan, 1997) has been found to be greater in obese adults, concomitant with decreased rates of FAO. In contrast, during alternate energy states, obese individuals have higher rates of FAO during steady state exercise of moderate intensity relative to participant's maximal aerobic capacity (Goodpaster, 2002; Horowitz & Klein, 2000; Perez-Martin, 2001). This reveals that obese individuals have the capacity to utilize fat as a fuel source, but the rate of utilization is altered in comparison to lean counterparts.

Although the relationship between FAO and obesity has been examined in body mass index (BMI) classification levels ranging from lean to obese, it is less clear what happens when obesity increases in severity. Previous studies have observed that an increase in BMI to classification levels of extreme obesity ( $\text{BMI} \geq 50 \text{ kg/m}^2$ ) related to a decrease in plasma FAO at rest and during exercise of moderate intensity (Thyfault, 2004), and also related to a decrease in FAO by skeletal muscle homogenates (Kim, 2000). In addition, Kim *et al* (2000) also found a relation between higher BMI levels and lower oxidative enzyme activities of carnitine palmitoyltransferase-1 and citrate synthase. However, none of these studies examined FAO in severely obese adults, and it remains unclear how this degree of obesity relates to FAO. In addition, these previous studies observed decrements in FAO at the level of the skeletal muscle and whole body, but did not directly relate if decreased FAO capacity of skeletal muscle explained observations of decreased systemic FAO at this level of obesity. Characterizing markers of skeletal muscle oxidative capacity and IMCL and systemic FAO will provide insight about factors that contribute to the etiology of obesity and its continued progression in severity of excess body fat accumulation, as well as help elucidate effective interventions to treat and prevent severe obesity and related conditions.

Currently, dietary induced weight loss and increased physical activity are the primary therapeutic modalities suggested for the prevention and treatment of obesity (Donnelly, 2009). The beneficial effects of weight loss induced by intentional energy restriction or bariatric surgery on improvements in insulin sensitivity and many other cardiometabolic risk factors are well documented (McTigue, 2003; Yanovski & Yanovski, 2002). Weight reduction by diet alone, however, has been reported not to improve FAO in obese at rest (Froidevux, 1993, Kelley, 1999; Schenk, 2009), or during exercise (Kanaley, 1993; Ranneries, 1998). This has been reported to



be consistent in the severely obese population; weight loss induced by caloric restriction did not correct impairments in FAO at rest or during exercise (Berggren, 2008; Schenk, 2009). Even more drastic weight loss induced by surgical techniques such as adjustable gastric banding and gastric bypass surgery has been shown to not improve FAO at rest (Galtier, 2006; Guesbeck, 2001) or during exercise (Thyfault, 2004). However, there is some evidence that the addition of aerobic exercise training to weight loss regimens improves FAO whether aerobic exercise training after bariatric surgery is performed for 10 days (Berggren, 2008), or aerobic training in combination with caloric restriction is carried out over 20 weeks (Schenk, 2009). Further research is clearly needed to examine how the addition of exercise interventions implemented with caloric restriction or surgical weight loss program can induce positive changes in FAO in severely obese adults.

## **1.1 PURPOSE**

The overall aims of this study are to profile skeletal muscle's FAO and storage capacity and systemic FAO across the BMI spectrum from normal weight through severely obese, sedentary adults, and to examine whether markers of skeletal muscle's capacity to oxidize fatty acids and fatty acid storage are related to systemic fat metabolism. In addition, this study will examine the effects of behavioral and surgical weight loss treatments with or without increased aerobic exercise on skeletal muscle's FAO and storage capacity and systemic FAO in severely obese participants. These studies will provide further insight into the potential role of FAO in the progression of obesity, as well as examine whether or not exercise is an important component of weight loss interventions to improve FAO in severely obese adults.

## 1.2 AIMS AND HYPOTHESES

**Aim 1.** To determine if skeletal muscle oxidative capacity and IMCL content are related to BMI among sedentary adults with weight status ranging from normal weight to severely obese. Further, to examine if systemic FAO at rest and during submaximal exercise in sedentary adults are associated with BMI.

**Hypothesis 1a.** Markers of oxidative capacity in skeletal muscle are associated with BMI, such that higher BMI relates to lower percent type 1 muscle fibers and succinate dehydrogenase (SDH), but higher IMCL.

**Hypothesis 1b.** Systemic FAO at rest and during exercise are associated with BMI, such that a higher BMI is associated with lower FAO at rest, but higher FAO during exercise of absolute and relative workloads.

**Aim 2.** To determine if skeletal muscle oxidative capacity and IMCL content are associated with systemic FAO at two physiological states (rest and during absolute and relative submaximal exercise) across the BMI spectrum.

**Hypothesis 2a.** Lower proportion of type I muscle fibers and SDH, and higher IMCL are associated with lower rates of FAO at rest and during exercise.

**Approach.** A cross sectional study was implemented to examine markers of skeletal muscle FAO capacity and utilization. Histochemical analysis of skeletal muscle biopsies was utilized to examine patterns of muscle fiber type distribution and SDH as markers oxidative capacity, and IMCL content. In addition, systemic FAO was measured with indirect calorimetry at rest and during steady state exercise at submaximal workloads.

**Aim 3.** To determine whether the addition of regular physical activity to weight loss regimens induced by either caloric restriction or bariatric surgery in severely obese, sedentary adults would

result in positive changes in skeletal muscle and systemic FAO at rest and during exercise compared to the effects of weight loss in the absence of increased physical activity.

**Hypothesis 3a.** Weight loss combined with exercise (i.e. diet with exercise or bariatric surgery with exercise) results in greater positive changes in markers of skeletal muscle oxidative capacity (i.e. percent type 1 muscle fibers and SDH) and greater negative changes in IMCL content compared to the weight loss by diet or weight loss by bariatric surgery alone.

**Hypothesis 3b.** Weight loss combined with exercise (i.e. diet with exercise or bariatric surgery with exercise) elicits greater positive changes in systemic FAO at rest and during exercise compared to the weight loss by diet or weight loss by bariatric surgery alone.

**Hypothesis 3c.** An increase in PA will relate to greater positive changes in markers of skeletal muscle oxidative capacity (i.e. percent type 1 muscle fibers and SDH), IMCL content and systemic FAO at rest and during exercise.

**Aim 4.** To determine if changes in skeletal muscle oxidative capacity and IMCL content induced by weight loss interventions are associated with changes in systemic FAO at rest and during submaximal exercise in previously sedentary, severely obese adults.

**Hypothesis 4a.** Changes in skeletal muscle markers of FAO capacity and neutral lipid content are associated with changes in systemic FAO at rest and during exercise.

**Approach.** This intervention study intended to examine the effects of weight loss with or without the addition of exercise by randomizing severely obese adults to one of the four weight loss interventions: 1) diet only, 2) diet plus exercise, 3) bariatric surgery only, and 4) bariatric surgery plus exercise. Measurement of skeletal muscle FAO capacity and IMCL content, as well

as systemic FAO at rest and during exercise, were assessed for change over the 6-month interventions and compared across intervention groups.

### **1.3 SIGNIFICANCE**

Obesity has reached epidemic levels in the United States and around the world. Impaired FAO has been reported to be associated with obesity and related metabolic disorders such as insulin resistance and type 2 diabetes. Current data suggest that key mechanisms underpinning obesity are a diminished FAO at rest associated with an excess accumulation of lipids within skeletal muscle. However, little is known about markers of skeletal muscle oxidative capacity and IMCL content, as well as systemic FAO, in severely obese adults who may have the greatest risk of morbidity and mortality. Characterizing FAO across the BMI spectrum will help to elucidate key factors that explain how FAO relates to obesity, particularly as the degree of obesity becomes more severe. Ultimately, these findings provide insight about appropriate interventions that can alleviate and prevent obesity and related disorders associated with accumulation of lipids within skeletal muscle.

In addition, this study will examine how changes in oxidative capacity and utilization of fat as a fuel source induced by weight loss differ by intervention. Chronic aerobic exercise training is a lifestyle habit known to prevent weight gain and improve the FAO capacity and utilization of fatty acids in normal weight adults. Much less is known about how physical activity affects fatty acid metabolism in obesity. The results of this study will provide novel information relevant to treating obesity with the most effective strategies. Specifically, this study will provide evidence as to whether physical activity in conjunction with behavioral and surgical weight loss

interventions can provide a positive stimulus on skeletal muscle and systemic FAO in severely obese adults.

## **2.0 REVIEW OF LITERATURE**

### **2.1 OBESITY EPIDEMIC**

Obesity is the result of an excess accumulation of body fat. The common clinical definition of obesity is based on the body mass index (BMI), a ratio of weight in kilograms to height in meters squared (Flegal, 2009). In adults, obesity is defined as a BMI greater or equal to 30.0 kg/m<sup>2</sup>. Additional subcategories of obesity classify individuals with class 1 obesity (BMI 30.0 – 34.9 kg/m<sup>2</sup>), class 2 obesity (BMI 35.0 – 39.9 kg/m<sup>2</sup>) and class 3 obesity (BMI ≥ 40.0 kg/m<sup>2</sup>) (National Institutes of Health, 1998; World Health Organization, 1997). The prevalence of obesity has increased throughout the past several decades. In comparison to 1980, the percentage of the United States' population classified as obese more than doubled from 15% to over 30% by 2004, and continues to increase (Ogden, 2006). The most alarming trend is the increase in the prevalence of class 3 obesity (Flegal, 2002; Freedman, 2002; Ogden, 2006; Strum, 2003).

As BMI increases from normal weight status to obese status, there is an increase in the risk of health problems that impair quality of life and threaten longevity (Bray, 2006; Calle, 1999). Diseases related to obesity affect almost every organ system, with the most prevalent comorbidity among those with a higher BMI being type 2 diabetes (Ogden, 2006). Class 3 obese individuals not only have the greatest risk of morbidity, but also the greatest risk of mortality (Calle, 1999). Fontaine *et al* (2003) estimated that a young adult white male with class 3 obesity

has 13 less years of life compared to his normal weight equivalent. The estimated health care costs for obese individuals are estimated to be \$1,419.00 per year more than those in the normal weight status (Finkelstein, 2009). Due to the disease burden and financial strain related to the obesity epidemic, understanding the etiology of this complicated disorder, as well as developing interventions that target mechanisms of obesity, are clinically relevant goal.

## **2.2 FATTY ACID OXIDATION**

Obesity is a multifactorial condition related to an interaction between genetics, behavior and the environment. Modifiable risk factors include overconsumption of nutrients and lack of physical activity. When energy intake exceeds energy expenditure, a positive energy balance results. The excess calories are partitioned to either oxidation or storage. It is hypothesized that the process of metabolizing fat is altered in obese; in particular, a decreased capacity for fatty acid oxidation (FAO) is suggested to be a key mechanism underpinning obesity. The excess fat is stored in adipose tissue and overflows into nonadipose organs. The accumulation of intracellular lipid in nonadipocytes can lead to lipotoxicity and compromise cellular functions such as insulin signaling (Pan, 1997). Because skeletal muscle utilizes a major proportion of substrates at rest and during exercise (Zurlo, 1990), a disruption of fat metabolism at the level of the skeletal muscle may be reflected at the level of the whole body. This impaired fat oxidation may exacerbate weight gain and the onset of obesity. Fat metabolism could be of central importance to the understanding of how to prevent and treat the epidemic of obesity and the related onset of disease, and is therefore the area of focus for this chapter.

### 2.2.1 Impaired Resting Fatty Acid Oxidation and Obesity

One possible determinant of obesity is a variation in substrate utilization. Lower FAO has been implicated as a predisposing risk factor of weight gain, as well as a metabolic consequence of the obese state. A prospective clinical study measured substrate utilization in a respiratory chamber over a 24-hour period, and found that a lower rate of fat oxidation predicted subsequent weight gain (Zurlo, 1990). Longitudinal studies using indirect calorimetry have since confirmed that lower FAO predisposes men and women to weight gain (Marra, 2004; Seidell, 1992), but others have failed to replicate the results (Katzmarzyk, 2000). A disturbance in fat metabolism also exists in obese state; cross sectional studies have characterized that all BMI classification levels of obesity have lower FAO. In particular, class 3 obese have lower FAO compared to lean, as well as overweight and class I obese counterparts (Hulver, 2003). These findings are supported by evidence from measurements of FAO *in vivo* by indirect calorimetry and tracer methodology (Colberg, 1995; Kelley, 1999; Thyfault, 2004), as well as *ex vivo* in muscle homogenates and muscle strips (Hulver, 2003; Kim, 2000). These later studies suggest that skeletal muscle may be the site of localization of the reduced capacity for FAO in obesity.

Skeletal muscle's capacity for fuel selection and utilization can be profiled by examining the composition and content of skeletal muscle across BMI classifications. Both noninvasive and invasive methods have been used to demonstrate that lipid content and accumulation of lipid metabolites (e.g. fatty acyl-CoA, diacylglycerol and ceramide) are increased in skeletal muscle of obese. *In vivo* imaging by computed tomography show a greater fat deposition within skeletal muscle among obese compared to lean (Kelley, 1991; Simoneau, 1995). Furthermore, studies of percutaneous muscle biopsy samples show that lipid content is greater in obese skeletal muscle (He, 2001; Goodpaster, 2001; Malenfant, 2001). The same method of examining



intramyocellular lipid (IMCL) content by immunohistochemical staining of muscle found that IMCL is even greater in extremely obese individuals than a group of normal weight individuals, as well as overweight and obese individuals (Hulver, 2003). Utilizing another technique for quantifying IMCL content, Hulver *et al* (2003) found that extremely obese participants tended to have greater rates of incorporating saturated fatty acids into intramuscular lipids compared to lean, overweight and obese groups. These studies present a consistent trend that lipid storage within skeletal muscle is higher with higher BMI.

The phenotype of obese skeletal muscle has been further characterized by fiber type distribution. Type 1 muscle fibers are the slow-twitch muscle fibers that have the highest oxidative enzyme activity (Lillioja, 1987). Type 2 muscle fibers are the fast-twitch muscle fibers that have the lowest oxidative capacity. Therefore, skeletal muscle fiber composition could influence an individual's metabolic and oxidative capacity. Several studies have demonstrated a significant, inverse relationship between percent type 1 muscle fibers and adiposity defined by either BMI (Hickey, 1995) or percent body fat (Helge, 1999; Lillioja, 1987; Wade, 1990). When using BMI as a discrete variable, evidence suggests that obese have fewer type 1, high oxidative muscle fibers than normal weight individuals. This association has been demonstrated in different muscle groups, including the rectus abdominus (Hickey, 1995; Tanner, 2002) and vastus lateralis muscle (Lillioja, 1987). Evidence accumulated from these studies indicates that fiber type distribution may relate to the etiology of obesity. To date, only one previous study completed a longitudinal analysis to examine this relationship, and ultimately found that a lower proportion of type 1 muscle fibers predicted a greater increase in body fat over a 19 year period (Karjalainen, 2006). Thus, fiber type composition, which can dictate the metabolic and oxidative profile of skeletal muscle, has been found to be associated with adiposity, and may

provide a possible explanation as to why obese subjects present with a diminished capacity to oxidize fatty acids. However, Helge *et al* (1999) found no relationship between muscle fiber type and fuel utilization at rest suggesting a disconnect between fiber type composition and FAO capacity.

Further support of impaired FAO in obese can be demonstrated by examining enzyme concentration and activity of skeletal muscle to describe the phenotype and metabolic health of skeletal muscle. For example, succinate dehydrogenase (SDH), a marker of skeletal muscle oxidative enzyme activity, was lower in obese subjects compared to lean subjects (He, 2001). Carnitine palmitoyl transferase (CPT), the rate-limiting enzyme responsible for the transfer of long-chain fatty acyl-CoA into the mitochondria, was lower in obese skeletal muscle compared to lean (Kelley, 1999; Kim, 2000; Simoneau, 1999). In addition, lower CPT activity was also associated with a decrease in fat oxidation. Citrate synthase, often used as a marker of mitochondrial content, has been shown to be lower in obese (Kim, 2000; Simoneau, 1999). Additional enzymes such as beta-hydroxyacyl CoA dehydrogenase trended toward being lower in obese compared to lean (Simoneau, 1999). Together, these findings suggest that obese individuals have lower oxidative enzyme activity, ultimately relating to the abnormalities in oxidative capacity of skeletal muscle in obese.

Another marker of skeletal muscle's oxidative capacity is the ability to deliver substrates to the tissue as estimated by capillary density or the capillary to muscle fiber ratio. Type 1 muscle fibers, the highly oxidative muscles with a high content of oxidative enzymes, have a higher capillary density than type 2 muscle fibers (Hudlicka, 1982). It would then be assumed that obese individuals who have a lower percentage of type 1 muscle fibers compared to lean, would then also have a lower estimate of capillary density. In fact, a decrease in the number of capillaries per muscle

fiber area has been previously reported in obese (Gavin, 2005; Lillioja, 1987), ultimately limiting the flux of substrates into and out of skeletal muscle and exacerbating the defects in the oxidative capacity of skeletal muscle. Kern (1999) also found that changes in capillary to fiber ratio related to changes in SDH activity, suggesting the relationship between capillarity and oxidative capacity of skeletal muscle.

### **2.2.2 Fatty Acid Oxidation in Obesity During Exercise**

Obese subjects may oxidize less fat in the postabsorptive, rested state compared to their lean counterparts, but what happens during altered metabolic states such as during exercise? Steffan *et al* (1999) found no significant differences in substrate utilization among obese and lean individuals during 15 min of treadmill walking at 50% and 70% of  $\text{VO}_{2\text{max}}$ . However, change in the mode or duration of the exercise protocol may influence the results. Perez-Martin *et al* (2001) compared substrate utilization of lean and overweight individuals while exercising on a cycle ergometer at intensities equating to 30, 40, 50 and 60% of theoretical maximal aerobic power. At all workloads, the overweight group had higher rates of fat oxidation compared to the lean group. Using indirect calorimetry, obese men and women have also been found to derive a greater proportion of energy from fat during submaximal exercise ( $\sim 50\% \text{VO}_{2\text{max}}$ ) compared to lean controls (Goodpaster, 2002; Horowitz & Klein, 2000). These studies employed tracer methodology to determine if the source of fat utilized during exercise was either circulating free fatty acids or nonplasma sources from adipose tissue or muscle. Both studies found that obese subjects relied more on nonplasma fatty acids during exercise of similar exercise intensities (Goodpaster, 2002; Horowitz & Klein, 2000). Among extreme obese subjects who completed 60 min of moderate intensity ( $\sim 50\% \text{VO}_{2\text{max}}$ ) on a cycle ergometer, there was no difference in total

lipid oxidation measured by indirect calorimetry, but determination of percent of plasma free fatty acid uptake oxidation measured by tracer methodology was significantly lower in the extreme obese compared to lean counterparts. When grouping all BMI categories, a significant negative correlation between BMI and percent plasma FFA uptake oxidized during exercise (Thyfault, 2004). Ultimately, these findings support the hypothesis that individuals with BMI classification levels ranging from overweight to class 3 obesity have higher rates of fat oxidation during exercise, and, in particular, utilize nonplasma sources of fatty acids instead of plasma free fatty acids. Taken together, it is likely that both the oxidative capacity and substrate availability from both plasma and intramuscular sources influence the overall substrate selection.

## **2.3 WEIGHT LOSS INTERVENTIONS FOR THE OBESE POPULATION**

Due to the suggested link between impaired fatty acid oxidation and the development of obesity and the related disorder of insulin resistance, it may be important to implement interventions that increase fat oxidation to treat and prevent obesity, as well as exert insulin-sensitizing effects on skeletal muscle.

The current recommendation for improving health outcomes that are clinically relevant is a weight loss of 5 to 10% of body weight (Jakicic, 2001; Stevens, 2006). There are several clinical and lifestyle therapies that induce weight loss, including diet therapy, physical activity and surgical therapy. Diet therapy typically involves a reduction in total energy intake, ranging from 500 to 1000 kcal/d, with less than 30% of total calories derived from fat. In addition to caloric restriction, most recommendations for weight loss include interventions incorporating changes in dietary intake and physical activity. The type of physical activity recommended by the American College of Sports

Medicine is a minimum of 150 min/wk of moderate-intensity physical activity (defined as 3.0 to 5.9 METs). In general, the greater amount of physical activity included in a weight loss program, the greater the energy deficit produced for greater weight loss. For example, the addition of such activity to caloric restrictions of 500-700 kcal/d results in a greater weight loss compared to a diet only intervention. However, exercise combined with greater caloric restriction (600-1000 kcal/d) results in weight loss similar to a diet only intervention (Donnelly, 2009; Jakicic, 2001).

Upon inducing weight loss, it is challenging to prevent weight regain. Five years after dietary interventions are implemented the average weight loss maintained is only about 3.2% below initial body weight. Although the literature emphasizes the importance of physical activity for weight loss maintenance, no study has examined the long-term effectiveness of physical activity for preventing weight regain (Anderson, 2001).

One weight loss intervention that has been shown to be successful at inducing clinically significant weight loss, as well as maintaining weight loss, is surgical treatment. This course of action is available to those individual with a BMI  $\geq 40$  kg/m<sup>2</sup> or those with a BMI  $\geq 35$  kg/m<sup>2</sup> in the presence of significant comorbidities (i.e. hypertension, dyslipidemia, diabetes, etc.). There are several options for surgical procedures, with the most common and conventional being Roux-en-Y gastric bypass (Brolin, 2002; Santry, 2005). This procedure combines gastric restriction and malabsorption by closing off the upper stomach to exclude digestion by the stomach, duodenum and the proximal jejunum (Brolin, 2002). Surgical treatment is often considered the most effective method for producing weight loss in severely obese individuals (Buchwald, 2004; Maggard, 2005). The initial weight loss in individuals who have bariatric surgery is ~30% of initial body weight, or a 20 to 30 kg decrease in body weight (Maggard, 2005). The average amount of weight loss by surgery is greater than weight loss induced by lifestyle modification (~10% of body weight). In

addition, the weight lost by surgical treatment is maintained for at least 10 years (Maggard, 2005); however, other studies have shown that the effectiveness of weight loss maintenance may be similar between the surgery and lifestyle interventions (Bond, 2009; Brolin, 2002). Regardless, the greater weight loss and promising outcome of maintaining the weight loss have led to the increasing popularity of surgical procedures completed in the severe obese population (Santry, 2005).

### **2.3.1 Weight Loss and Fat Metabolism in Obesity**

Although long-term caloric restriction almost always results in weight loss it does not always reconcile the impairment in fat oxidation at rest (Froidevaux, 1993; Kelley, 1999), or during exercise (Kanaley, 1993). Even more substantial weight loss induced by bariatric surgery does not seem to improve rates of systemic FAO. This has been supported by examining rates of FAO with labeled palmitate in skeletal muscle homogenates; after considerable weight loss from surgery, previously morbid obese adults had rates of FAO comparable to extremely obese participants (Berggren, 2008). During exercise, previously severely obese subjects following weight loss from surgery had suppressed FAO during steady state, submaximal exercise at absolute and relative workloads compared to matched controls (Guesbeck, 2001).

Due to the reduction in fat mass, fat oxidation may further decline in obese losing weight (Franssila-Kallunki, 1992; van Aggel-Leijssen, 2001). The lower rate of fat oxidation in post obese is indicative of weight regain (Froidevaux, 1993). These results indicate that impaired capacity to utilize fatty acids in obese individuals may be indicative of the obese state rather than an adaptation response. This may also indicate that loss of excess adipose tissue induced by dietary therapy and bariatric surgery may not effect the characteristics of skeletal muscle that are associated with the impairment of fat oxidation in obese. Weight loss induced by caloric restriction was found to have

no effect of muscle fiber type proportion or cross sectional areas for each type of fiber. However, there was an increase in muscle fiber capillarization for each muscle fiber type (estimated by capillary/fiber ratio, capillary density) and an increase in SDH activity in all fiber types (indicative of increased muscle oxidative capacity) (Kern, 1999).

It has been suggested that weight loss alone may not enhance fat oxidation due to the loss in fat free mass that accompanies weight loss. Fat-free mass, especially skeletal muscle, is the primary tissue to utilize fat at rest and moderate intensity, steady state exercise. Because exercise may help to maintain fat-free mass, it is suggested that weight loss with exercise may lead to a favorable improvement in fat metabolism. This hypothesis, however, has never been directly tested.

### **2.3.2 Exercise Intervention and Fat Metabolism in Lean and Obese Adults**

Compared to caloric restriction, exercise alone is less efficient in attaining a negative energy balance to induce weight loss. However, the impact of exercise on fat oxidation has a beneficial impact on fat balance, leading to additional benefits for enhanced insulin sensitivity. Fat utilization during exercise is dependent on intensity and duration of exercise, as well as the individual's fitness level and substrate stores. Low to moderate power outputs primarily rely on the metabolism of fat as a fuel source. The fat source available during exercise includes plasma fatty acids and triacylglycerol and fat stored in skeletal muscle - intramyocellular lipid (IMCL) content. Increase in exercise intensity leads to an increasing reliance on carbohydrates as an energy source. Although the utilization of fat has decreased, the total amount of fat burned is similar to lower to moderate intensity exercise if one takes into consideration the percentage of fat oxidized for a higher rate of energy expenditure at high intensities. In regards to duration of exercise, evidence suggests that an increase in duration during

steady state, submaximal exercise will derive energy from fat rather than carbohydrates (Stefanick, 1993).

Exercise training in lean individuals increases fat oxidation at rest and during exercise. Evidence from cross-sectional studies suggest that endurance trained athletes have greater rates of fat oxidation at rest compared to sedentary, lean controls (Romijn, 1993). Additionally, trained individuals also oxidize more fat during prolonged, steady state exercise at low and moderate intensities in comparison to untrained individuals, (Klein, 1994; Turcotte, 1992). Studies implementing exercise interventions in previously sedentary adults also show improvements in fat oxidation during submaximal exercise bouts on a cycle ergometer (Friedlander, 2007; Hurley, 1986). Another training study using a single-leg model, demonstrated that 8 weeks of training resulted in higher respiratory quotient after 60 min of submaximal prolonged exercise, thus indicating higher levels of fat oxidation in the trained leg compared to the untrained leg (Kiens, 1993). These results provide evidence that endurance training has the capacity to improve fat oxidation at rest and during submaximal exercise in normal weight individuals.

Several mechanisms may be responsible for inducing changes in fat oxidation in response to endurance training. For example, muscle fiber type patterns may be a determining factor for utilization of fatty acids. It is known that type I muscle fibers are the fiber type primarily activated during moderate intensity exercise due to the greater oxidative capacity. Training stimulates an increase in the proportion of type I to type II muscle fibers. This was demonstrated in a study comparing the muscle fiber composition of trained subjects who had higher proportion of type I fibers and lower proportions of type II muscle fibers than untrained subjects (Jansson & Kaijser, 1987). Exercise training also induces changes in the activity of mitochondrial enzymes involved in the oxidation of fat. The rate limiting steps of fat oxidation have been identified as the transport of



fatty acids across the plasma membrane, transport across the mitochondrial membrane and oxidative capacity. Compared to untrained, lean controls, endurance trained subjects have higher activity of the oxidative enzymes citrate synthase and  $\beta$ -hydroxyacyl-CoA dehydrogenase (Helge, 2006; Jansson & Kaijser, 1987; Stisen, 2006). If obese individuals could accrue the same benefits from exercise training as normal weight adults, then such an intervention might improve fat oxidation, and/or decrease toxic lipids stored in skeletal muscle.

However, application of exercise training programs prescribed to sedentary lean individuals, do not necessarily result in the same improvements in fat oxidation among obese individuals. High intensity endurance training (70 to 75% of  $VO_{2max}$ ) did not produce any changes in fat oxidation in obese individuals (Kanaley, 2001; Van Aggel-Leijssen, 2002). However, obese men and women completing a low-intensity exercise training program (~40%  $VO_{2max}$ ) on the cycle ergometer for 12 weeks, 3 days per week had a decrease in respiratory exchange ratio during exercise, indicating increased fat oxidation (van Aggel-Leijssen, 2001; van Aggel-Leijssen, 2002). Exercise training at a similar workload also increased rate of fat oxidation during steady state exercise after only 4 weeks of training 5 days per week (Venables & Jeukendrup, 2008). Future studies incorporating exercise training programs for increasing fat oxidation in obese need to take into consideration the duration, frequency and intensity of the physical activity. No study found an improvement in resting fat oxidation after completing an exercise intervention (Saris & Schrauwen, 2004; Van Aggel-Leijssen, 2001; Van Aggel-Leijssen, 2002).

Few studies have examined mechanisms for change in fat oxidation in obese who completed an exercise training program, but Bruce *et al* (2006) found that changes in the activity of the oxidative enzymes citrate synthase and  $\beta$ -hydroxyacyl-CoA dehydrogenase followed patterns similar to those found in endurance trained, lean individuals. Improvement of oxidative

capacity after exercise training in obese participants was also demonstrated in the study conducted by Dube *et al* (2008). Previously sedentary, overweight and obese adults participated in moderate intensity exercise for 45 min per session at least 4 days per week for a total of 16 weeks. After completing the intervention, an increase in skeletal muscle succinate dehydrogenase (SDH) activity (indicative of mitochondria activity), percentage of type I slow oxidative fibers and capillary density increased (Dube, 2008). Overall this evidence suggests that exercise training can induce similar changes in metabolism of skeletal muscle of obese that occur in lean individuals.

### **2.3.3 Exercise in Combination with Weight Loss in Obese Adults**

General recommendations for overweight and obese individuals are to induce weight loss by energy restriction and physical activity. Exercise training in combination with hypocaloric diets improved systemic FAO as measured by indirect calorimetry in previously sedentary, obese individuals at rest (Schenk, 2009; Solomon, 2008; van Aggel-Leijssen, 2001) and during exercise (van Aggel-Leijssen, 2001). These improvements in fat oxidation were observed after 12 weeks of exercise performed at 40% of  $\text{VO}_{2\text{max}}$  for 1 hour, 4 times per week (van Aggel-Leijssen, 2001).

There is also evidence to suggest that incorporation of physical activity with surgery induced weight loss is associated with greater weight loss. Physical activity assessed by questionnaire was a significant predictor of weight loss following gastric bypass surgery (Welch, 2008). When gastric bypass surgery patients self-reported achieving at least 200 min/wk of moderate intensity physical activity postoperatively, greater weight loss was experienced for up to 12 months compared to those who do not meet these minimum requirements (Evans, 2007). Authors of both studies suggest that the results of their findings are limited, and there is a need for a randomized study to establish a

definitive link between physical activity and postoperative weight loss and maintenance (Evans, 2007; Welch, 2008). It is less clear whether endurance exercise training has any additional effects, particularly in the setting of surgery induced weight loss. Few studies have examined how exercise in postoperative individuals can improve fat oxidation. Berggren *et al* (2008) used a cross-sectional study design to compare fat oxidation in post-obese individuals 12 months after surgery to severe obese and lean sedentary individuals after 10 days of training at about 75% of  $\text{VO}_{2\text{peak}}$ . Analysis of stable isotope tracer measurements revealed that fat oxidation was similar in extreme obese and previously, severe obese patients; both groups had lower rates of fat oxidation than lean subjects (Berggren, 2008). No study has examined how long-term, exercise endurance program in postoperative patients relates to changes in substrate metabolism in this population. A limitation of studies that compare exercise and energy restriction interventions is that participants who exercise are also more likely to maintain a restricted caloric intake. Compliance to the intervention may confound results.

Only one previous study has examined the combined effects of physical activity and dietary composition. Physical inactivity induced by 60 h of bed rest combined with either an isocaloric high fat or high carbohydrate diet significantly increased skeletal muscle fat. However, the addition of acute exercise bouts in combination with the high fat diet prevented a significant increase in skeletal muscle fat. The effects of combined physical activity with a high carbohydrate diet were not examined (Stettler, 2005). The results of this study suggest that regardless of dietary composition, exercise may prevent the accumulation of fat stored within the skeletal muscle.

### 3.0 METHODS

#### 3.1 SUBJECTS AND SCREENING

Participants for this study were recruited from two ongoing studies entitled Preventing Adverse Effects of Class II and Class III Obesity (RENEW – **Re-Energize with Nutrition, Exercise and Weight loss**) and Physical Activity following Surgery-Induced Weight Loss (POWER). Details of the RENEW randomized trial for weight loss interventions are presented by Goodpaster *et al* (2010). In brief, RENEW sought to assess the effect of lifestyle interventions on adverse health risks in severely obese adults. Class 2 and class 3 obese ( $\text{BMI} \geq 35.0 \text{ kg/m}^2$ ) adults were recruited for a one year behavioral weight management intervention that involved randomization to either a diet plus exercise or an identical diet intervention with the exercise program delayed for 6 mo.

An ancillary study to the main RENEW trial was performed in a subset of severely obese and control participants who consented to participate in the additional testing. The participants involved in the intervention groups were asked to sign a separate informed consent that involved participation in additional testing at baseline and at the 6 month follow up time point, whereas the control participants only completed additional testing at baseline. Recruitment goals were to enrollment of a total of 60 subjects, including 20 class 2 and 3 obese, and 20 normal weight ( $\text{BMI} 20 \text{ to } 25 \text{ kg/m}^2$ ) and 20 class 1 obese ( $\text{BMI} 30 \text{ to } 34.9 \text{ kg/m}^2$ ) controls matched for age,

race/ethnicity and gender. All RENEW participants were recruited for the current study's cross-sectional analysis examining specific aims 1 and 2, and intervention participants were also incorporated into the current study's 6 month intervention study used to examine specific aims 3 and 4. For the intervention study, the RENEW participants were already randomized to the two intervention groups of the RENEW parent study, and recruitment for the ancillary study relied on the randomization process to distribute equal number of participants from both interventions.

An additional 20 participants from the POWER study were also included in the current study's analysis for the randomized intervention study. The primary purpose of this study was to determine if physical activity provided additional benefits after the initial three-month weight loss from bariatric surgery. The study sought to examine this aim by enrolling patients following the initial three month weight loss from bariatric surgery. The 6 mo interventions included a control group that received monthly health education classes or a physical activity regimen. The current study sought to incorporate 20 participants from POWER, 10 participants having been randomized to the health education group and 10 participants randomized to the bariatric surgery plus exercise group. The addition of POWER participants brings the combined total of participants in the current study to 40 volunteers who are participating in one of four interventions used to examine aims 3 and 4.

To be eligible for RENEW subjects were to be between the ages of 30 to 55 years, and to be eligible for POWER, subjects were to be between the ages of 21 and 60 years. Subjects of either gender, and all race and ethnic groups, were eligible for participation in this study. The BMI of eligible subjects for the RENEW control group was 20.0 to 24.9 kg/m<sup>2</sup> for the normal weight group and 30.0 to 34.9 kg/m<sup>2</sup> for the class 1 obese group. Severely obese subjects in the RENEW intervention had to have a BMI  $\geq$  35.0 kg/m<sup>2</sup>. In addition, RENEW participants could

not have been enrolled in a formal weight loss program or study, nor lost more than five percent of current body weight in the previous 6 months. In regards to the POWER study, there was no BMI criteria; it was only required that the participants had undergone bariatric surgery.

To determine eligibility for the intervention, potential participants came in for a screening visit consisting of the informed consent process, measurement of height and weight, a health history and physical with a study physician, and a fasting blood draw. Fasting blood analysis included CBC and platelets, creatine, liver function with liver enzymes, lipid panel, fasting glucose and insulin, and thyroid stimulating hormone (TSH). Any values that fell outside the normal range were excluded. All women of childbearing potential completed a urine pregnancy test to determine pregnancy. Women could not be pregnant, or plan on becoming pregnant.

All potential RENEW intervention participants obtained written medical clearance from a primary care physician (PCP). POWER participants were included if a study physician provided written approval to participate in regular physical activity; this approval was not only based on medical examination but also results of electrocardiogram (EKG) recorded during rest and the maximal exercise test. The step of obtaining medical clearance excluded adults for which the intervention would not be safe.

### **3.2 EXPERIMENTAL PROCEDURES**

If a research volunteer met all eligibility criteria and requirements, the individual was scheduled for the baseline experimental procedures to be conducted within 2-3 visits. The normal weight and class I obese subjects underwent baseline measurements only, whereas the intervention participants (class II and III obese) completed post-intervention measurements at approximately

6 months in addition to baseline measurements. Intervention participants undergoing bariatric surgery completed baseline testing one to three months following date of surgery. Delaying baseline testing and initiation of the intervention gave way to initial weight loss following bariatric surgery, but it also allowed for recovery after surgery so that the experimental procedures and intervention could be accomplished and still allowed for detection of changes in primary outcome variables. For this reason, baseline BMI of participants who underwent bariatric surgery may appear lower than the severe obese weight status required to undergo this medical procedure. The time frame for the intervention study, including the experimental procedures, was approximately 8 months: Baseline testing during weeks -4 to 0, intervention during weeks 0 to 24), and post test during weeks 24 to 28. The following procedures were conducted at the Endocrinology and Metabolism Research Center (EMRC) of the University of Pittsburgh: anthropometrics and body composition, energy expenditure, muscle biopsy and analysis, exercise testing and physical activity assessment.

### **3.2.1 Anthropometrics and Body Composition**

Body weight was measured on a calibrated, digital scale (Tanita Corporation of America, Inc., Arlington Heights, IL), while height was measured with a standard scale. Measurements were taken until 2 measurements were within  $\pm 0.1$  units.

Dual-energy x-ray absorptiometry (DXA) was used to measure fat mass (FM) and fat free mass (FFM), and also measure changes in these components upon completion of intervention. These variables when used to determine body fat (BF) as a percentage of total body weight. Although this exposes participants to radiation, the amount of radiation exposure received from a DXA scan is about 0.002 rem. This amount of radiation is a small part (0.3 rem) of the average whole body radiation exposure that each member of the public receives per year from radiation exposure that is recognized as being totally free of the risk of causing genetic defects (cellular

abnormalities) or cancer. The risk associated with the amount of radiation exposure received from this procedure is considered to be low and comparable to other everyday risks. The DXA unit that was used during this study was a General Electric Lunar prodigy scanner with EnCore software 2005 (General Electric Healthcare, Madison, WI, USA). The scanner was calibrated at the beginning of each day to maintain accurate values. Participants were asked to remove all metal accessories and clothing; a hospital gown was provided if necessary. The measurement was completed as prescribed by the manufacturer; whole body scans were acquired with the subject supine and aligned with scanner table.

### **3.2.2 Resting Fatty Acid Oxidation**

Indirect calorimetry was used to determine respiratory exchange ratio (RER), and quantify systemic fatty acid oxidation (FAO) before and after intervention. RER is the ratio of the volume of carbon dioxide ( $\text{CO}_2$ ) produced to oxygen ( $\text{O}_2$ ) consumed, and is theorized to range from 0.7 to 1.0. A lower RER is indicative of utilizing fat as a fuel source, where as a higher RER is characteristic of carbohydrate oxidation (Stefanick, 1993).

Following an overnight fast, a 30 minute measurement was made using an open canopy system (Parvo Medics, Salt Lake City, UT). Prior to testing, calibration of both gas and pressure was completed. Subjects were tested in the morning between the hours of 7:00AM and 10:00AM. Subjects sat or laid comfortably for 20 minutes prior to measuring. Subjects were told to refrain from fidgeting and sudden movements. This test was performed for 30 minutes in order to estimate carbohydrates and fat oxidation from expired rates of  $\text{O}_2$  ( $\text{VO}_2$ ) and  $\text{CO}_2$  ( $\text{VCO}_2$ ). The first five minutes of data collection was discarded due to lack of reliability. The average rates of resting FAO and carbohydrate oxidation (CO) were measured during 20 minutes



of steady state. The following equations developed by Frayn (1983) was used to determine rates of resting FAO and CO in g/min:

$$\text{CO} = 4.55\text{VCO}_2 - 3.21\text{VO}_2 - 2.87$$

$$\text{FAO} = 1.67\text{VO}_2 - 1.67\text{VCO}_2 - 1.92$$

Rates of FAO were also expressed relative to kg of FFM and percent of energy derived from fat (EF).

### **3.2.3 Physical Activity Assessment**

Participants wore a multisensory activity monitor (SenseWear Pro3, BodyMedia, Pittsburgh, PA) to provide an objective measure of physical activity (PA) during free-living conditions and monitor the effect of interventions. All participants were asked to wear the armband at baseline and after the 6-month intervention for seven to eleven consecutive days. Data were analyzed by the armband's software (Innerview Software version 5.1 for aims 1 and 2 and Innerview Software version 6.1 for aims 3 and 4) with the specific intensity threshold value for PA set at  $\geq 3.0$  METs. Only days in which the participant wore the armband for 85% of a 24-hour period were accepted as valid days. Participants must have at least 3 days of valid days to be included in analysis. For each valid day, the sum of the number of minutes of PA per day was determined. The minutes of PA per day for all valid days were then divided by the number of valid days analyzed. The daily average was then multiplied by 7 to express PA as minutes per week (min/wk).

### 3.2.4 Peak Aerobic Capacity Test

During baseline testing only, subjects performed a graded exercise test to determine peak aerobic capacity ( $\text{VO}_{2\text{peak}}$ ) and the workload for the submaximal exercise tests. Electrocardiograms and blood pressure response were monitored to determine the safety of the subjects during exercise testing. Previous research in the EMRC has used a modified Balke protocol that was adapted for sedentary, lean and severely obese participants on a cycle ergometer (Excalibur, Lode, The Netherlands). For normal weight and class 1 obese subjects, the workload started at 25 Watts (W) for women and 50 W for men, and then increased by 25 W every two minutes. For intervention subjects, the intensity level began at 25 W for two minutes and increased 15 W every two minutes. Subjects breathed through a mouthpiece connected to a two way breathing valve (Hans Rudolph, Kansas City, MO) during the test, and expired air was collected into a mixing chamber interfaced to a computerized metabolic cart (Moxus, AEI Technologies, Naperville, IL) to measure expiratory flow and  $\text{VO}_2$  and  $\text{VCO}_2$  fractions. The metabolic cart analyzed the data for  $\text{VO}_2$  every 20 seconds. The criterion for termination was voluntary exhaustion. A resulting test was considered a maximal effort if two or more of the following criteria were met: a) RER equal or greater to 1.15, b) maximal heart rate equal or greater to their age predicted maximal heart rate ( $220 - \text{age}$ ), or c) a plateau in the  $\text{VO}_{2\text{peak}}$  curve. Heart rate, electrocardiography, rate of perceived exertion and blood pressure were recorded at rest, every 2 min during testing, and for at least 5 minutes during recovery. After termination of the test each subject cooled down by pedaling at a light resistance for 2 min and then were asked to be seated until their heart rate and blood pressure returned to normal resting values. Individuals were excluded from further participation if signs and/or symptoms occurred prior to the test or during exercise testing, and then referred to their PCP for further care. The exercise test was stopped if the subject had any of the following: a) a positive ECG ( $> 2\text{mm}$  ST segment depression), b) signs or symptoms of cardiovascular decompensate (hypotensive response to exercise), c) onset of angina or angina like symptoms, d) shortness of breath, e) change in heart rhythm, or f) signs of

of poor perfusion (light-headedness). The EMRC was equipped with cardiac emergency equipment and if an emergency develops a code C was called to hospital emergency staff for immediate medical response.

### **3.2.5 Submaximal Exercise Tests**

An absolute submaximal exercise bout was added to the study protocol after three participants had completed the study, thus the sample size for this analysis is 31, including 9 normal weight, 7 class 1 obese and 15 severely obese adults. Subjects performed 20 min of exercise on a cycle ergometer after a 10 to 12 hour fast; the first 10 min were performed at an absolute workload of 25 W, and the next 10 min were performed at 60% of their pre-determined  $\text{VO}_{2\text{peak}}$ . This test quantified rates of absolute and relative FAO and EF during exercise using indirect calorimetry as performed during the graded exercise test. This test was conducted at baseline, and after intervention at the same absolute workloads to examine how intervention effects fat oxidation during exercise.

At least one week following the  $\text{VO}_{2\text{peak}}$  test, subjects were asked to maintain their habitual diet and will be given instructions to complete a 3-day food record prior to the submaximal exercise test. It has been shown by previous research that recent diet effects substrate oxidation. This diary consists of each research subject writing down on a piece of paper everything they ate and drank for three days. All subjects replicated the same diet at post testing. They were instructed to avoid strenuous physical activity for two days prior to the study. Subjects were asked to record food intake in a diary for the three days prior to this exercise bout so that they can replicate their diet during the three days preceding the post exercise-training bout of exercise.

### **3.2.6 Muscle Biopsy**

A muscle biopsy was performed at baseline in all participants incorporated in the cross sectional study, and before after interventions in those participants included in the intervention study. The following were the primary outcome variables: distribution of types of muscle fiber, oxidative enzyme capacity and neutral lipid content. Subjects were instructed not to perform physical exercise 48 h prior to the muscle biopsy procedure to help prevent acute effects of exercise on muscle metabolism. In addition, participants were asked to fast for 12 h prior to the morning of the appointment. A study physician performed a percutaneous muscle biopsy of the vastus lateralis at the CTRC (Clinical Translation Research Center, University of Pittsburgh Medical Center, Montefiore Hospital, Pittsburgh, PA). Muscle biopsies were obtained from the middle region of the vastus lateralis muscle, about 15 cm above the patella, and approximately 2 cm away from the fascia by the percutaneous needle-biopsy technique. A quarter-sized area in the mid-thigh was anesthetized with 11 ml of 2% buffered lidocaine. A 1.5 cm long incision was made with a scalpel to insert a Bergstrom needle into the vastus lateralis muscle. Suctioning was applied to the needle core to obtain approximately 100-150 mg tissue. If this failed to provide an adequate muscle sample, the participant was asked for voluntary permission for a second pass through the same site. The incision was closed with sterile tapes, and a topical antibiotic and compressive dressing applied for a period of 24 hours. This procedure took approximately 15 minutes, was relatively painless, and well-tolerated.

Specimens were trimmed of excess adipose tissue and blotted dry. Portions of the tissue sample were mounted on a small piece of cork with mounting medium (Miles, Inc., Elkhart, IN), placed into isopentane cooled at -160°C by liquid nitrogen for two to three minutes, and then

stored in a plastic container which was placed into liquid nitrogen. Samples were stored at -80°C for until analysis.

Histochemical analysis of the percutaneous muscle biopsies was used to estimate the following variables: 1) muscle fiber type distribution, i.e. percent of type 1 (high oxidative) muscle fibers using enzymatic staining of ATPase activity, 2) oxidative capacity using an enzymatic staining procedure for succinate dehydrogenase (SDH) and 3) neutral lipid content using a quantitative Oil Red O staining procedure to estimate intramyocellular lipid (IMCL) content. Histochemical analyses were performed on serial sections. The samples were sectioned into 10 µm pieces on a cryostat (Cryotome E; Shandon Scientific) at -20°C and placed on glass slides. For the cross sectional study, for to five participants were sectioned onto the same slide and were analyzed together to minimize staining bias. For the prospective, longitudinal study, pre and post intervention samples of the same participant were placed on individual glass slides. Images were visualized using a Leica microscope (Leica DM 4000B; Leica Microsystems) and digitally captured (Retiga 2000R camera; Q Imaging), and analyzed using specialized software (Norther Eclipse, v6.0; Empix Imaging). For analysis of intensity of staining (SDH and IMCL), three to four images of sections were captured in 16-bit grayscale and averaged.

The immunohistochemical staining with antibodies specific to type 1 and type 2A muscle fibers allows for determination of percentage of type 1 muscle fibers. Signals for specific fibers were recorded using a flourescein isothiocyanate excitation filter and a TRITC excitation filter.

Oxidative capacity of skeletal muscle was determined with SDH staining. A stock solution was made with 10 ml of 0.1 M PBS, 10 ml of 0.3% nitro blue tetrazolium, 4 ml of 0.065% KCN, 4 ml of 0.47% MgCl, and 8 ml of distilled water. The working solution was made by adding 2 ml of the stock solution to 200 µl of 1 M sodium succinate and 2–3 drops of 0.5%

menadione. Slides were incubated for 45 min at room temperature and then washed in distilled water (3 times). The slides were then postfixed in 4% formaldehyde for 10 min, washed in distilled water, and mounted. Quantification of SDH staining was performed using image analysis of staining intensity.

IMCL content was determined by staining neutral lipid (mainly triacylglycerol) with Oil Red O soluble dye. Oil Red staining was quantified as the difference in positive staining intensity from background. An average IMCL content for each muscle fiber type (i.e. type 1 and type 2) was calculated, as well as average IMCL content for total muscle fibers measured.

### **3.3 RANDOMIZATION AND GROUPING FOR INTERVENTION STUDY USED TO EXAMINE AIMS 3 AND 4**

All class 2 and 3 research volunteers recruited from RENEW were randomized into one of the following groups: diet only or diet combined with physical activity for six months. The Physical Activity and Weight Management Research Center of the University of Pittsburgh implemented the interventions. All POWER subjects undergoing bariatric surgery were randomized into health education group or bariatric surgery combined with physical activity. This intervention was implemented by the EMRC. The analysis compared two different methods of weight loss (dietary caloric restriction and bariatric surgery) with and without exercise in severely obese. Although the interventions were different, the study could still examine changes in systemic and skeletal muscle FAO at rest and during exercise.

### **3.3.1 Diet Only Intervention**

Details of the dietary intervention without physical activity for 6 months were previously described (Goodpaster, 2010). Subjects in this group were prescribed an energy restricted dietary intervention that has been shown to effectively reduce body weight by 8-10% within the 6 months of treatment (Jakicic, 1999; Jakicic, 2003; Jakicic, 2008). Energy intake was reduced to 1200 to 2100 kcal/d based on initial body weight. For those participants with an initial body weight of < 200 pounds, a total caloric intake of 1200-1500 kcal/d was prescribed. Participants weighing 200 to 250 pounds were prescribed a 1500-1800 kcal/d diet, and those >250 pounds were prescribed a total caloric intake of 1800-2100 kcal/d. The macronutrient composition recommended consisted of 20-30% dietary fat intake, 50-55% carbohydrate intake, and 20-25% protein intake. To facilitate adoption to the dietary recommendations, individuals were provided with meal plans, a calorie counter book and meal replacements. Participants were asked to monitor eating behaviors by recording dietary intake in a weekly diary that was reviewed by intervention staff.

### **3.3.2 Diet and Exercise Intervention**

Subjects in this group received a weight loss program that initiates physical activity modifications in addition to the dietary recommendations. These participants were prescribed the same energy restricted diet as described above for the diet only intervention. In addition, this group was assigned an exercise program based on recommendations from the American College of Sports Medicine suggests that 60 min/d on most days of the week (300 min/wk) maximizes weight loss and minimize weight regain. Specifically, subjects were instructed to engage in

moderate intensity exercise five days per week. Exercise progressed in a gradual manner (5-10 min/d in 4 week intervals) in an attempt to maximize adherence and minimize the onset of musculoskeletal injury. The total duration began at 20 min/d, if able, and gradually progressed to at least 60 min/d. The recommended mode for the exercise program was walking. However, walking could be substituted with swimming, bicycling, or other similar exercises. Participants self-monitored exercise behaviors in a weekly diary that was reviewed by intervention staff.

### **3.3.3 Bariatric Surgery and Health Education Intervention**

This group of participants served as the control arm. To retain interest and compliance with in this group, participants were involved in a total of 6 health education sessions. These sessions were held 1 time per month in the EMRC Conference Room. The sessions lasted approximately 1 hour. The sessions provided up-to-date information and covered relevant topics such as medication use, nutrition, upper body stretching, and communicating with health care professionals. Instructors included study staff.

### **3.3.4 Bariatric Surgery and Exercise Intervention**

Subjects were asked to participate in three to five exercise sessions weekly. The total duration began with a duration that was feasible to complete a session set at an intensity equal to 60 to 70% of maximal heart rate. Over the first 3 months, exercise gradually progressed to at least 120 min/wk. Participants were able to access facilities with stationary cycle ergometers and treadmills and were provided supervision by a member of the study staff. Recommended modes of exercise outside the facility included walking or cycling outdoors. Each participant in the



group was provided with a heart rate monitor and instructed in how to use the rate of perceived exertion scale to monitor exercise intensity. Participants self-monitored exercise behaviors in a weekly diary that was reviewed by intervention staff.

### **3.4 DATA ANALYSIS**

The Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL) was used to compute the appropriate descriptive statistics and perform the statistical analyses, with significance level set at  $p < 0.05$ . All assumptions of statistical testing were not violated unless otherwise noted. For multivariate regression diagnostics, normality of residuals was tested with the Shapiro-Wilk test. The assumptions of linearity and homoscedasticity were assessed by visual analysis of residual plots. The assumption of collinearity was assessed by examining the collinearity statistics - tolerance and variance inflation factor. For descriptive purposes, the frequencies (N) and percentages (%) of categorical data and medians with 25<sup>th</sup> and 75<sup>th</sup> percentiles for continuous variables are presented.

#### **3.4.1 Statistical Analysis for Cross Sectional Study (Aims 1 and 2)**

Descriptive statistics are presented for the entire sample and by BMI classifications (normal weight, NW; BMI: 18.0-24.9 kg/m<sup>2</sup>; class 1 obese, O; BMI: 30.0-34.9 kg/m<sup>2</sup>; and class 2 and 3, or severely obese, SO; BMI  $\geq 35.0$  kg/m<sup>2</sup>) where appropriate. Relationships between categorical subject characteristics (gender and race) and BMI were tested with the Wilcoxon-signed rank sum test, whereas relationships between continuous subject characteristics (age, height, weight, FFM, FM, BF, PA, absolute and relative VO<sub>2peak</sub> and percentage of VO<sub>2peak</sub> during both

submaximal workloads) and BMI were tested with Pearson correlation. Multivariable linear regression was used to examine the relationship between BMI and markers of skeletal muscle oxidative capacity (percent type 1 muscle fibers and SDH), IMCL content by muscle fiber type and total muscle fibers measured and systemic FAO at rest and during exercise (RER, absolute and relative FAO and EF; Table 1), controlling for age, gender, race, cardiovascular (CV) fitness (relative  $\text{VO}_{2\text{peak}}$ ) and PA. The percentage of  $\text{VO}_{2\text{peak}}$  was included as a covariate in the multivariate regression model examining BMI and systemic FAO at the relative submaximal workloads if the relationship was found to be significant.

**Table 1.** Variables and units of skeletal muscle markers of oxidative capacity, intramyocellular lipid (IMCL) and systemic fatty acid oxidation (FAO).

Variable	Abbreviation	Units
Skeletal muscle markers of oxidative capacity in postabsorptive state		
Type 1 muscle fiber	% type 1	%
Succinate dehydrogenase	SDH	AU
Intramyocellular lipid (IMCL) in postabsorptive state		
Of type 1 muscle fiber	IMCL/type 1	AU
Of type 2 muscle fiber	IMCL/type 2	AU
Of total muscle fiber	IMCL/total	AU
Systemic fatty acid oxidation (FAO) at postabsorptive rest and during absolute or relative submaximal exercise		
Respiratory exchange ratio	RER	
Absolute FAO		mg/min
Relative FAO		mg/kgFFM/min
Energy derived from fat	EF	%

AU – arbitrary unit; mg/min – milligrams per minutes; mg/kgFFM/min – milligrams per kilograms of fat free mass per minute.

Per aim 2, Pearson correlation was applied to examine whether markers of skeletal muscle oxidative capacity and IMCL content were correlated with systemic FAO at rest and during exercise.

### 3.4.2 Statistical Analysis for Longitudinal Intervention Study (Aims 3 and 4)

Eighteen participants enrolled in the RENEW substudy were randomized to the diet only (D) and diet with exercise (DE) groups. Of the seven participants randomized to the D group, three completed the 6-month intervention. The D group was not included in the following analysis due to the unexpected randomization imbalance leading to insufficient sample size. Of the 11 participants randomized to the DE group, all completed the 6-month intervention. Participants enrolled in the POWER study were included in the current study if a participant's dataset was complete by the time of data analysis. This resulted in the inclusion of eight participants in bariatric surgery with health education (S) group and eight participants in bariatric surgery with exercise (SE) group, bringing the total sample size to 27. Differences between baseline categorical subject characteristics (i.e. gender and race) and intervention groups were tested with the Chi Square test, whereas differences in continuous subject characteristics (i.e. baseline age, height, weight, BMI, FFM, FM, BF, PA, absolute and relative  $\text{VO}_{2\text{peak}}$ ) in intervention groups were examined using Kruskal-Wallis nonparametric analysis of variance (ANOVA). Although intention-to-treat analysis was performed (i.e. intervention assignment was used as the independent variable in all analyses regardless of intervention compliance), Kruskal-Wallis nonparametric ANOVA was also used to examine differences in change in weight (measured as percent body weight lost) and change in PA from baseline to post-intervention by intervention groups.

Multivariable linear regression was used to examine whether intervention group was associated with changes in skeletal muscle oxidative capacity, IMCL and systemic FAO at rest and during exercise, after controlling for age, gender, race, baseline BMI, CV fitness, PA and percent of body weight lost. Changes in percentage of type 1 fibers, RER at rest, and RER, absolute FAO and EF during the relative submaximal workload were log transformed to meet

assumption of normality. Preliminary analysis revealed a majority of the models were not statistically significant, in part because none of the covariates were significantly related to changes in skeletal muscle oxidative capacity, IMCL or systemic FAO at rest and during exercise. Therefore, analyses were redone to test whether intervention group was associated with changes in skeletal muscle oxidative capacity, IMCL and systemic FAO at rest and during exercise only controlling for percent weight lost. The percentage of  $\text{VO}_{2\text{peak}}$  was included as a covariate in the multivariate regression model examining systemic FAO at the relative submaximal workloads across intervention groups if the relationship was found to be significant. Multivariate linear regression was also used to examine how PA in the total sample (regardless of intervention group) related to changes in *ex vivo* and *in vivo* FAO, controlling for percent of body weight lost. These analyses were performed in the eight participants in the S group and the eight participants in the SE group who were matched to eight participants in the DE group so that there were no differences across groups in age, gender and race. The three participants removed from the DE group were one male African American (BMI: 38.3 kg/m<sup>2</sup>), two female African Americans (BMI: 47.1 and 52.0 kg/m<sup>2</sup>). The final sample size included 24 participants.

Per aim 4, Pearson correlation was used to examine whether changes in skeletal muscle oxidative capacity and IMCL were correlated to changes in systemic FAO variables at rest and during exercise.

### **3.4.3 Power Analysis**

For the proposed cross sectional study designed to examine relationships between BMI and markers of skeletal muscle oxidative capacity, IMCL content and systemic FAO, a sample size of 31 achieves 80% power to detect an R-Squared of 0.13 attributed to BMI when controlling for five additional independent variables with an R-Squared of 0.4 using an F-test with a significance level set at  $p < 0.05$ . Per aim 2, a sample size of 31 achieves 80% power to detect an  $r$  of 0.48 using a two-sided hypothesis with a significance level of 0.05.

For the proposed longitudinal study designed to examine whether intervention group was associated with changes in FAO, a sample size of 24 achieves 80% power to an R-Squared of 0.26 attributed to 2 independent variables (indicating groups 1 and 2 in comparison to group 3), adjusting for an additional 2 independent variables with an R-Squared of 0.2 using an F-test with a significant level  $p < 0.05$ . Per aim 4, a sample size of 24 achieves 80% power to detect an  $r$  of 0.54 with a significance level of 0.05.

## **4.0 RESULTS**

### **4.1 PARTICIPANTS OF CROSS SECTIONAL STUDY**

Characteristics of participants in the cross sectional study (Aims 1 and 2) are presented for the entire sample and by body mass index (BMI) classifications for descriptive purposes (Table 2). The majority of participants were middle aged, Caucasian females with low cardiovascular (CV) fitness. By study design, participants were either normal weight (NW; 26%), class 1 obese (O; 21%) or severely obese (SO; 53%). All participants self-reported their physical activity level as sedentary. The activity monitors revealed that the average minutes of moderate and vigorous physical activity per week was indicative of a physical active lifestyle defined by the American College of Sports Medicine and American Heart Association (Haskell, 2007; Table 2).

Distribution of BMI was significantly different among Caucasians and African Americans ( $p=0.01$ ) such that African Americans had higher BMI values than Caucasians. BMI was significantly positively related to fat free mass (FFM;  $r=0.55$ ,  $p<0.001$ ), fat mass (FM;  $r=0.97$ ,  $p<0.001$ ) and percentage body fat (BF;  $r=0.88$ ,  $p<0.001$ ), and negatively related to PA ( $r=-0.41$ ,  $p=0.02$ ) and CV fitness (relative peak oxygen consumption,  $VO_{2peak}$ ;  $r=-0.65$ ,  $p<0.001$ ). Gender ( $p=0.64$ ), age ( $p=0.06$ ), height ( $p=0.13$ ), weight ( $p=0.79$ ) and absolute  $VO_{2peak}$  ( $p=0.42$ ) were not significantly related to BMI.

**Table 2.** Characteristics by BMI classification and for the total sample of participants in the cross sectional study. Presented in medians and quartiles unless otherwise noted.

	<b>Normal Weight</b> (n=9)	<b>Class 1 Obese</b> (n=7)	<b>Severely Obese</b> (n=18)	<b>Total</b> (n=34)
Female (n, %)	8 (88.9)	7 (100)	15 (83.3)	30 (88.9)
Caucasian (n, %)	8 (88.9)	6 (85.7)	8 (44.4)	22 (88.2)
Age (yr)	46 (35, 48)	50 (47, 54)	49 (46, 50)	48 (46, 51)
Height (cm)	162.7 (160.5, 172.4)	162.7 (159.8, 165.8)	162.5 (159.2, 166.2)	162.5 (159.2, 170.3)
Weight (kg)	61.8 (58.4, 63.6)	86.0 (81.3, 94.2)	122.0 (112.9, 126.2)	101.7 (71.1, 123.2)
BMI (kg/m <sup>2</sup> )	22.6 (21.4, 23.3)	32.2 (31.4, 32.5)	43.8 (41.3, 48.8)	37.6 (24.5, 44.0)
FFM (kg)	37.1 (36.1, 43.3)	42.8 (41.8, 47.1)	54.5 (50.5, 59.2)	49.7 (41.8, 55.0)
FM (kg)	18.8 (17.6, 20.8)	37.0 (35.0, 39.8)	59.0 (55.3, 66.1)	44.9 (22.4, 60.1)
BF (%)	32.0 (26.5, 35.3)	42.7 (42.1, 45.6)	52.3 (49.2, 54.7)	44.1 (38.0, 52.6)
PA (min/wk)	818.4 (647.9, 944.1)	685.7 (631.0, 822.6)	498.1 (269.3, 720.8)	656 (408, 898)
VO <sub>2peak</sub> (l/min)	2.0 (1.8, 2.2)	2.2 (2.2, 2.4)	2.1 (1.8, 2.2)	2.1 (1.8, 2.3)
CV Fitness (VO <sub>2peak</sub> , ml/kgFFM/min)	50.2 (48.2, 53.5)	52.1 (50.7, 53.1)	36.7 (32.6, 40.5)	41.7 (36.0, 51.3)

BMI – body mass index; FFM – fat free mass; FM – fat mass; BF – body fat; PA - physical activity; VO<sub>2peak</sub> – peak aerobic capacity; CV Fitness – cardiovascular fitness

## 4.2 MARKERS OF SKELETAL MUSCLE OXIDATIVE CAPACITY AND INTRAMYOCELLULAR LIPID CONTENT ACROSS BMI SPECTRUM

The proportion of type 1 muscle fiber (% type 1), succinate dehydrogenase (SDH) enzyme content and intramyocellular lipid (IMCL) content by each muscle fiber type area and total muscle fibers measured are shown in Table 3. BMI was not significantly related to any markers of skeletal muscle FAO capacity or measure of IMCL content in univariate or multivariate analyses (Table 3).

**Table 3.** Markers of skeletal muscle fatty acid oxidative (FAO) capacity and intramyocellular lipid (IMCL) content by body mass index (BMI) classification and for total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between markers of skeletal muscle FAO capacity and IMCL content with predictor variables in unadjusted and adjusted models are also presented.

	<b>Normal Weight</b> (n=9)	<b>Class 1 Obese</b> (n=7)	<b>Severely Obese</b> (n=18)	<b>Total</b> (n=34)	<b>BMI Unadjusted</b>	<b>BMI Adjusted*</b>
Markers of skeletal muscle FAO capacity						
% Type 1	54.9 (46.4, 58.5)	50.7 (44.6, 57.0)	57.0 (42.7, 62.0)	54.4 (44.0, 61.9)	p=0.49	p=0.25
SDH (AU)	36.2 (35.2, 37.4)	34.9 (34.2, 35.6)	35.4 (33.5, 37.9)	35.5 (33.6, 37.1)	p=0.79	p=0.74
IMCL						
Of type 1 muscle fibers (AU)	3.0 (2.7, 3.4)	3.6 (3.0, 4.8)	4.4 (3.7, 5.0)	3.8 (3.0, 4.8)	p=0.35	p=0.70
Of type 2 muscle fibers (AU)	1.9 (1.7, 2.3)	2.2 (1.7, 2.6)	2.8 (2.4, 3.7))	2.4 (2.4, 3.3)	p=0.09	p=0.27
Of total muscle fibers (AU)	5.1 (4.5, 5.9)	5.8 (4.8, 7.7)	7.4 (5.9, 8.4)	6.2 (4.8, 8.3)	p=0.18	p=0.47

\*BMI adjusted multivariate analysis controlled for the following covariates: age, gender, race, cardiovascular (CV) fitness (relative  $\text{VO}_{2\text{peak}}$ ) and PA.



### 4.3 SYSTEMIC FATTY ACID OXIDATION AT REST AND ACROSS BMI SPECTRUM

Systemic fatty acid oxidation (FAO) at rest expressed as respiratory exchange ratio (RER), absolute or relative FAO, or energy derived from fat (EF) in the total sample of participants and by BMI classifications are shown in Table 4, in addition to the associations between BMI and systemic FAO at rest in the total sample of participants. BMI was not significantly associated with any expressions of the systemic FAO at rest in univariate analyses, nor in multivariate regression analyses (Table 4). Age, gender, race, CV fitness and PA were also not significantly associated with any expressions of systemic FAO in the multivariate analyses.

**Table 4.** Systemic fatty acid oxidation (FAO) at rest by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of systemic FAO with predictor variables in unadjusted and adjusted models are also presented.

	<b>Normal Weight</b> (n=9)	<b>Class 1 Obese</b> (n=7)	<b>Severely Obese</b> (n=18)	<b>Total</b> (n=34)	<b>BMI Unadjusted</b>	<b>BMI Adjusted*</b>
RER	0.79 (0.78, 0.81)	0.79 (0.76, 0.84)	0.79 (0.77, 0.81)	0.79 (0.77, 0.81)	p=0.17	p=0.66
Absolute FAO (mg/min)	71.8 (65.1, 76.8)	74.3 (54.7, 97.3)	96.0 (81.4, 108.6)	83.5 (61.8, 98.5)	p=0.34	p=0.28
Relative FAO (mg/kgFFM/min)	1.8 (1.7, 2.0)	1.7 (1.3, 2.0)	1.8 (1.4, 2.0)	1.8 (1.4, 2.0)	p=0.24	p=0.96
EF (%)	68.6 (61.6, 72.0)	69.6 (51.5, 80.8)	68.6 (60.5, 74.1)	68.6 (59.2, 75.3)	p=0.08	p=0.43

\*BMI adjusted multivariate analysis controlled for the following covariates: age, gender, race, cardiovascular (CV) fitness (relative  $\text{VO}_{2\text{peak}}$ ) and PA.

#### 4.4 METABOLIC RESPONSES DURING ABSOLUTE WORKLOAD OF SUBMAXIMAL INTENSITY

The metabolic responses during the absolute workload (25 W) are presented in Table 5 by BMI classification and in total sample of participants along with correlations between BMI and metabolic responses. Absolute  $\text{VO}_2$  (l/min) had a large, positive association with BMI ( $r=0.77$ ,  $p<0.001$ ), but this relationship was not statistically significant when expressing  $\text{VO}_2$  relative to FFM ( $p=0.08$ ). Exercise intensity expressed as percent of relative  $\text{VO}_{2\text{peak}}$  (ml/kgFFM/min) had a large, positive association with BMI ( $r=0.75$ ,  $p<0.001$ ), suggesting that participants with higher BMI were exerting a greater effort relative to their maximal capacity to complete this exercise bout compared to participants with lower BMI.

**Table 5.** Metabolic responses during the absolute submaximal workload (25 W) by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between these variables and BMI are also presented.

	<b>Normal Weight (n=9)</b>	<b>Class 1 Obese (n=7)</b>	<b>Severely Obese (n=15)</b>	<b>Total (n=31)</b>	<b>Pearson correlation, p-value</b>
$\text{VO}_2$					
l/min	0.7 (0.66, 0.72)	0.8 (0.7, 1.1)	1.0 (0.8,1.2)	0.8 (0.7, 1.0)	$r=0.77$ $p<0.001$
ml/kgFFM/min	16.9 (16.3, 19.2)	17.2 (15.3,25.4)	18.0 (15.9,22.6)	17.7 (16.0,19.4)	$p=0.08$
$\text{VO}_{2\text{peak}}$ (%)					
ml/kgFFM/min	34.8 (30.6,37.8)	34.9 (30.4,49.8)	50.2 (46.9,60.4)	41.0 (34.5,53.1)	$r=0.75$ $p<0.001$

$\text{VO}_2$  - expired rates of  $\text{O}_2$ ;  $\text{VO}_{2\text{peak}}$  - peak aerobic capacity

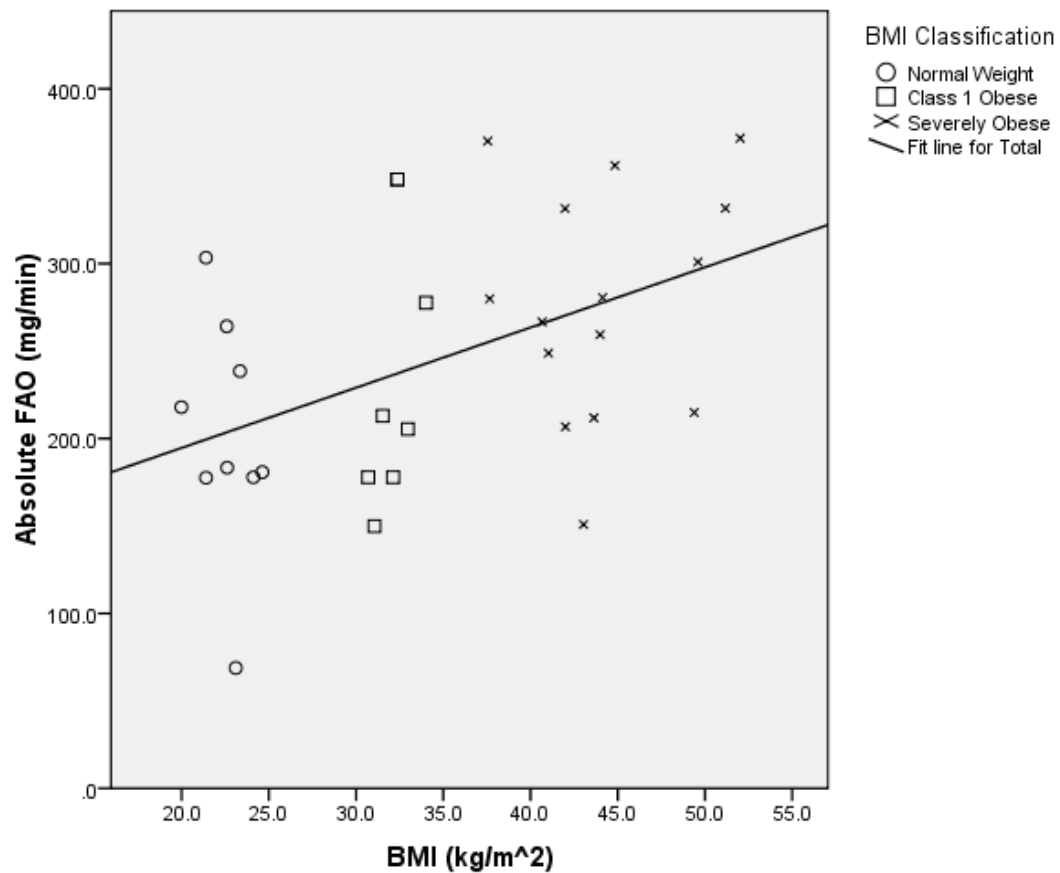
#### 4.5 SYSTEMIC FATTY ACID OXIDATION ACROSS BMI SPECTRUM DURING ABSOLUTE WORKLOAD OF SUBMAXIMAL INTENSITY

Systemic FAO measured during the absolute submaximal workload is presented in Table 6 along with the associations between systemic FAO and BMI. BMI was not statistically significant in univariate or multivariate analysis when FAO was expressed as RER, relative FAO or EF while completing a submaximal exercise bout at the same absolute resistance. Higher BMI was significantly associated with higher absolute FAO during the exercise bout set at 25 W ( $p<0.01$ ; Figure 1), and explained 21% of the variance of absolute FAO at the absolute workload. In the multivariate model controlling for potential confounders, BMI was significantly associated with absolute FAO ( $p<0.01$ ), but the model was not significant ( $p=.06$ ; Table 6).

**Table 6.** Systemic fatty acid oxidation (FAO) measured during the absolute submaximal workload by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of FAO with predictor variables in unadjusted and adjusted models are also presented.

	<b>Normal Weight</b> (n=9)	<b>Class 1 Obese</b> (n=7)	<b>Severely Obese</b> (n=15)	<b>Total</b> (n=31)	<b>BMI Unadjusted</b>	<b>BMI Adjusted*</b>
RER	0.84 (0.78, 0.84)	0.84 (0.82, 0.85)	0.83 (0.85,0.85)	0.83 (0.82,0.85)	$p=0.29$	$p=0.37$
Absolute FAO (mg/min)	183.4 (177.9, 238.5)	209.2 (177.9, 295.3)	279.9 (231.9,331.6)	243.7 (181.4,302.8)	$r^2=0.21$ $p<0.01$	$p<0.01$
Relative FAO (mg/kgFFM/min)	5.0 (4.4, 6.0)	4.4 (3.9, 6.8)	5.0 (4.3,5.8)	5.0 (4.1,6.0)	$p=0.43$	$p=0.05$
EF (%)	51.1 (49.8, 69.9)	50.7 (47.8, 56.8)	50.4 (47.1, 4.7)	50.8 (46.7,58.0)	$p=0.24$	$p=0.93$

\* multivariate analysis controlled for the following covariates: age, gender, race, cardiovascular (CV) fitness (relative  $VO_{2peak}$ ) and PA.



**Figure 1.** Absolute fatty acid oxidation (FAO) during the absolute submaximal workload across by BMI ( $r=0.46$ ,  $p<0.01$ ).

#### 4.6 SYSTEMATIC FATTY ACID OXIDATION ACROSS BMI SPECTRUM DURING RELATIVE SUBMAXIMAL EXERCISE BOUT

The metabolic responses during the workload relative to 60%  $\text{VO}_{2\text{peak}}$  are presented in Table 7 along with the correlations between the metabolic responses and BMI. Absolute  $\text{VO}_2$  (l/min) was

not statistically significantly related to BMI ( $p=0.10$ ), but  $VO_2$  relative to FFM had a large negative association with BMI ( $r=-0.75$ ;  $p<0.001$ ). Although all participants were supposed to perform the relative submaximal bout at the same intensity, there was a moderate negative association between BMI and exercise intensity as assessed by percentage of relative  $VO_{2peak}$  used to complete the relative submaximal exercise bout ( $r=-0.36$ ,  $p=0.04$ , respectively). This suggests that participants with higher BMI were not exerting as much relative effort during the exercise bout. To minimize the effect of this discrepancy, the multivariate model controlled for percent of relative  $VO_{2peak}$  measured during the exercise bout.

**Table 7.** Systemic fatty acid oxidation (FAO) measured during the absolute submaximal workload by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of FAO with predictor variables in unadjusted and adjusted models are also presented.

	<b>Normal Weight</b> (n=9)	<b>Class 1 Obese</b> (n=7)	<b>Severely Obese</b> (n=15)	<b>Total</b> (n=31)	<b>Pearson correlation, p-value</b>
$VO_2$					$VO_2$
l/min	1.3 (1.1,1.6)	0.8 (0.7, 1.1)	1.0 (0.8,1.2)	1.3 (1.2, 1.4)	$p=0.10$
ml/kgFFM/min	32.9 (30.1,34.5)	17.2 (15.3,25.4)	18.0 (15.9,22.6)	25.6 (22.4,33.1)	$r=-0.75$ $p<0.001$
$VO_{2peak}$ (%)					
ml/kgFFM/min	64.1 (62.3,66.2)	64.4 (60.2,65.3)	60.8 (58.1,64.1)	62.3 (59.9,65.3)	$r=-0.36$ $p=0.04$

- expired rates of  $O_2$ ;  $VO_{2peak}$  - peak aerobic capacity

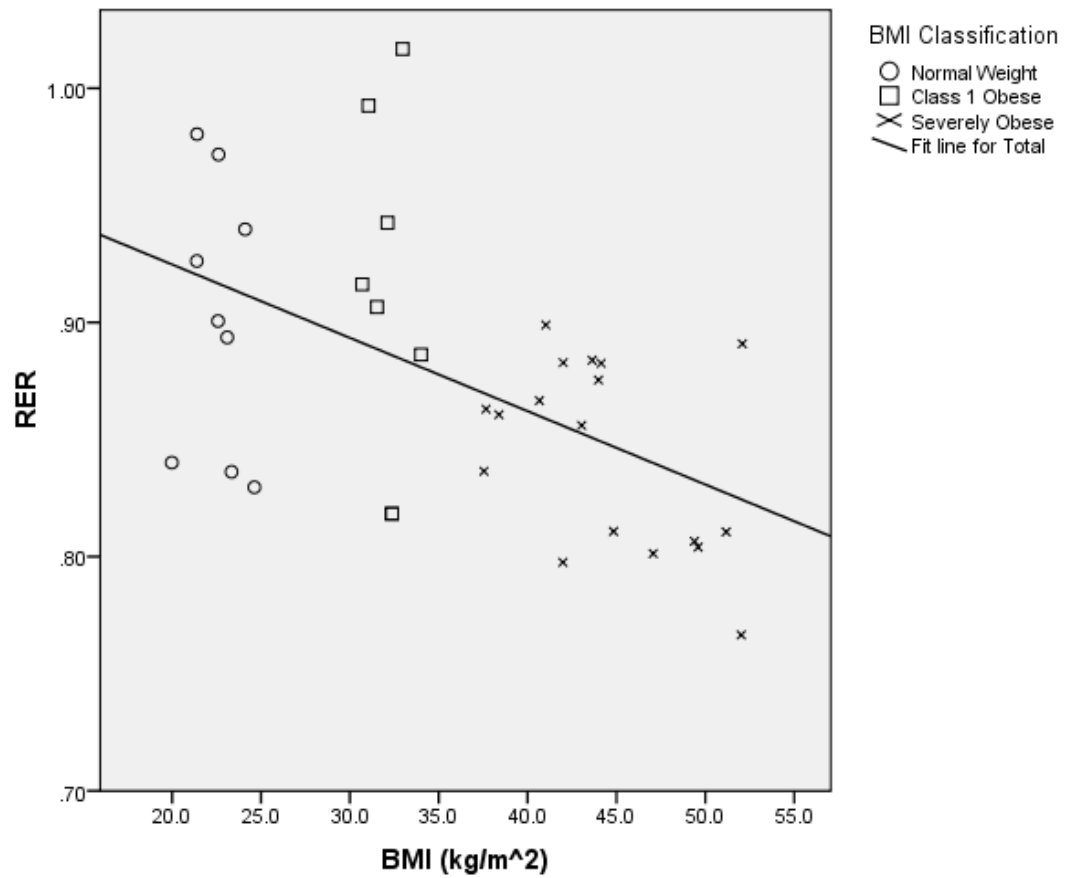
Systemic FAO measured during the relative submaximal workload is presented in Table 8 in addition to relationships between systemic FAO and BMI. In the univariate analysis, BMI was significantly associated with FAO during relative submaximal workload when expressed as

RER ( $r=-0.54$ ,  $p<0.01$ ; Figure 2), absolute FAO ( $r=0.45$ ,  $p<0.01$ ; Figure 3) and EF ( $r=0.55$ ,  $p<0.01$ ; Figure 4). However, when controlling for potential confounding variables in the multivariate analysis, BMI only retained significant association with absolute FAO, such that BMI explained 29% of the variance in RER ( $p<0.01$ ; Table 9). A 5-unit increase in BMI ( $\text{kg/m}^2$ ) was associated with a 0.1 unit increase in RER (Table 9). BMI was not significantly related to relative FAO during this submaximal exercise bout set at 60% of  $\text{VO}_{2\text{peak}}$  in the univariate ( $p=0.18$ ) or multivariate analyses ( $p=0.10$ ).

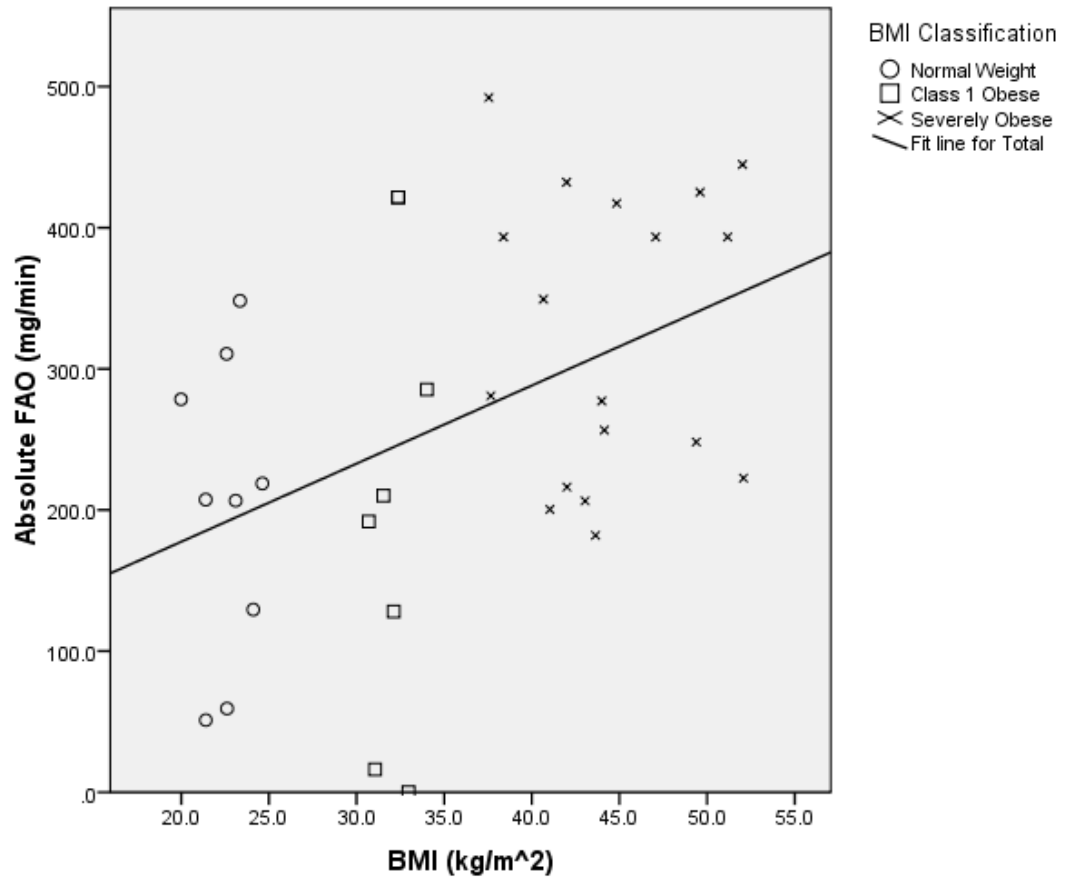
**Table 8.** Systemic fatty acid oxidation (FAO) measured during the relative submaximal workload by body mass index (BMI) classification and in total sample of participants in the cross sectional study. Presented in medians and quartiles. Relationships between expressions of FAO with predictor variables in unadjusted and adjusted models are also presented.

	<b>Normal Weight</b> (n=9)	<b>Class 1 Obese</b> (n=7)	<b>Severely Obese</b> (n=15)	<b>Total</b> (n=31)	<b>BMI Unadjusted</b>	<b>BMI Adjusted*</b>
RER	0.90 (0.84, 0.94)	0.91 (0.87, 0.96)	0.86 (0.81, 0.88)	0.887 (0.82, 0.91)	$r^2=0.29$ $p<0.01$	$p<0.01$
Absolute FAO (mg/min)	207.4 (129.4, 278.5)	201.0 (100.1, 319.3)	315.1 (229.0, 411.3)	256.6 (200.4, 393.5)	$r^2=0.45$ $p<0.01$	$p=0.10$
Relative (mg/kg/min)	5.2 (3.3, 6.5)	4.3 (2.0, 7.4)	5.3 (4.6, 7.2)	5.2 (4.0, 7.2)	$p=0.18$	$p=0.10$
EF (%)	29.6 (17.5, 49.6)	26.3 (13.0, 39.9)	44.7 (36.0, 60.9)	37.7 (27.8, 57.1)	$r^2=0.30$ $p<0.01$	$p=0.07$

\* multivariate analysis controlled for the following covariates: age, gender, race, cardiovascular (CV) fitness (relative  $\text{VO}_{2\text{peak}}$ ) and PA.

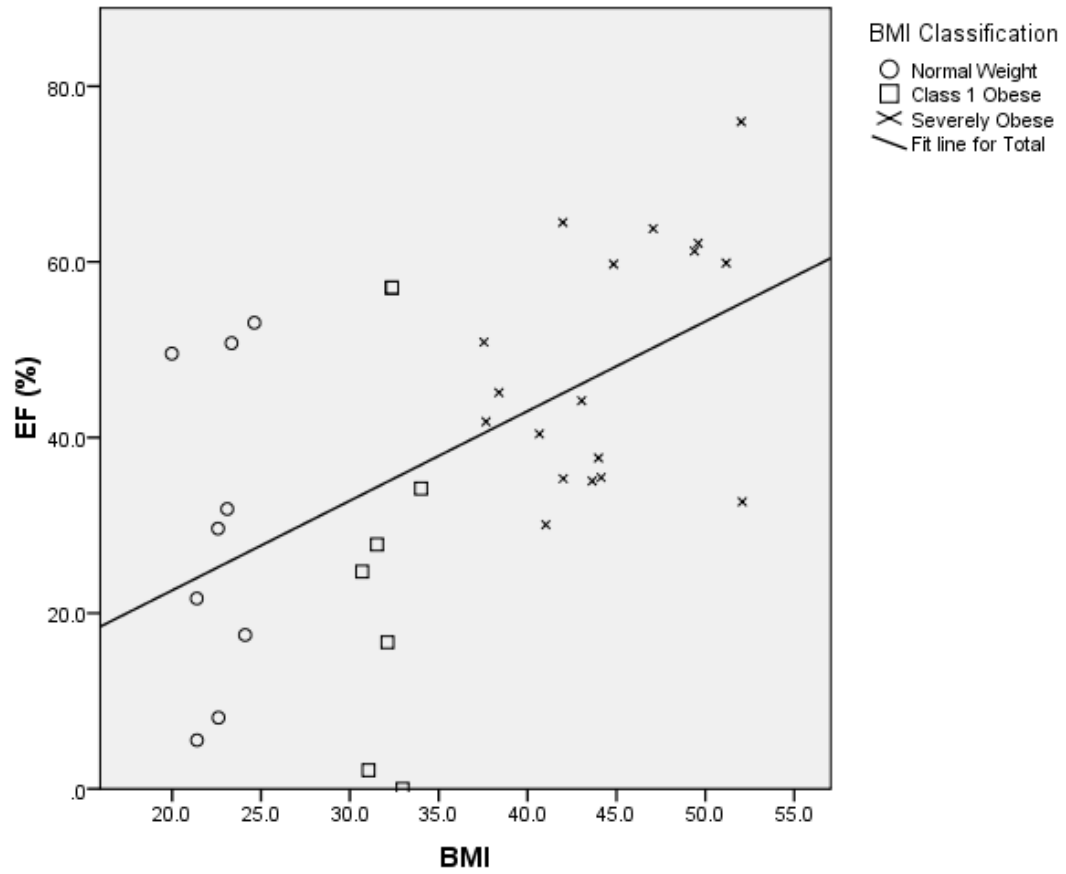


**Figure 2. Respiratory exchange ratio (RER) during the relative submaximal workload across by BMI**  
**( $r=-0.54$ ,  $p<0.01$ ).**



**Figure 3.** Absolute fatty acid oxidation (FAO) during the relative submaximal workload across by BMI ( $r=0.45$ ,  $p<0.01$ ).





**Figure 4.** Energy derived from fat (EF) during the relative submaximal workload across by BMI ( $r=0.55$ ,  $p<0.01$ ).

**Table 9.** Multivariable models testing the relationships between BMI and absolute fatty acid oxidation (mg/min) during a submaximal relative workload, controlling for potential confounders (N=34).

	Beta (SE)	p value
Intercept	-358.34 (563.54)	0.53
BMI (kg/m <sup>2</sup> )	8.48 (3.12)	0.01
Age (yr)	-1.58 (3.71)	0.67
Gender (reference: male)	-150.52 (61.34)	0.02
Race (reference: Caucasian)	59.08 (55.81)	0.30
CV Fitness (VO <sub>2peak</sub> , ml/kgFFM/min)	2.20 (3.96)	0.58
PA (min/wk)	0.07 (0.06)	0.20
Percentage of VO <sub>2peak</sub> (ml/kgFFM/min)	5.69 (5.02)	0.27

**Model  $r^2=0.42$   
 $r^2$  from BMI=0.21**

#### **4.7 RELATIONSHIP BETWEEN SKELETAL MUSCLE AND SYSTEMIC FATTY ACID OXIDATION**

Correlations between systemic FAO at rest and markers of skeletal muscle FAO and IMCL content are presented in Table 10. Higher IMCL content in type 1 muscle fibers ( $r=0.35$ ,  $p=0.04$ ), type 2 muscle fibers ( $r=0.48$ ,  $p<0.01$ ), and total muscle fibers measured ( $r=0.46$ ,  $p<0.01$ ) were significantly, moderately related to higher absolute FAO at rest. No other relationships between IMCL content were significantly related to systemic FAO at rest,

including expression of FAO as RER, relative FAO or EF (Table 10). No other relationships between systemic FAO at rest and makers of skeletal muscle FAO and IMCL content were statistically significantly.

**Table 10.** Correlations between systemic fatty acid oxidation (FAO) at rest and skeletal muscle oxidative capacity and intramyocellular (IMCL) content. Presented correlation coefficients/p values.

	RER	Absolute FAO (mg/min)	Relative FAO (mg/kgFFM/min)	EF (%)
Type 1 muscle fibers (%)	p=0.25	p=0.68	p=0.34	p=0.32
SDH (AU)	p=0.96	p=0.24	p=0.71	p=0.70
IMCL in type 1 muscle fibers (AU)	p=0.41	r=0.35 p=0.04	p=0.71	p=0.69
IMCL in type 2 muscle fibers (AU)	p=0.26	r=0.48 p<0.01	p=0.45	p=0.43
IMCL in total muscle fibers (AU)	p=0.28	r=0.46 p<0.01	p=0.53	p=0.51

SDH – succinate dehydrogenase, RER – respiratory exchange ratio, FAO – fatty acid oxidation, EF – energy derived from fat

There was no significant association between systemic FAO at the absolute submaximal workload and markers of skeletal muscle FAO and IMCL content (data not shown). However, there was a significant moderate association between percent type 1 muscle fibers and absolute FAO during the relative submaximal workload ( $r=0.35$ ,  $p=0.04$ ) suggesting that higher percent type 1 muscle fibers related to higher absolute FAO during submaximal exercise relative to 60% of  $VO_{2peak}$ . Although not statistically significant, there was possible evidence to suggest that

percent type 1 muscle fibers may be negatively correlated to RER ( $r=-0.33$ ,  $p=0.06$ ) and positively correlated to relative FAO ( $r=0.29$ ,  $p=0.09$ ) and EF ( $r=0.33$ ,  $p=0.05$ ) during this workload (Table 11). The other marker of skeletal muscle oxidative capacity, SDH, was not significantly associated to any expression of systemic FAO during exercise this exercise bout, nor was IMCL content (Table 11). Percentage of type 1 muscle fibers was not significantly related to any other systemic FAO measurement during rest or exercise at the absolute or relative submaximal workloads (Table 11).

**Table 11.** Correlations between systemic fatty acid oxidation (FAO) during the relative submaximal exercise bout and skeletal muscle oxidative capacity and IMCL content. Presented correlation coefficients/p values.

	RER	Absolute FAO (mg/min)	Relative FAO (mg/kgFFM/min)	EF (%)
Type 1 muscle fibers (%)	$r=-0.33$ $p=0.06$	$r=0.35$ $p=0.04$	$r=0.29$ $p=0.09$	$r=0.33$ $p=0.05$
SDH (AU)	$p=0.78$	$p=0.25$	$p=0.45$	$p=0.80$
IMCL in type 1 muscle fibers (AU)	$p=0.88$	$p=0.59$	$p=0.54$	$p=0.96$
IMCL in type 2 muscle fibers (AU)	$p=0.41$	$p=0.14$	$p=0.81$	$p=0.42$
IMCL in total muscle fibers (AU)	$p=0.74$	$p=0.27$	$p=0.81$	$p=0.70$

SDH – succinate dehydrogenase, RER – respiratory exchange ratio, FAO – fatty acid oxidation, EF – energy derived from fat

#### **4.8 SUBJECT CHARACTERISTICS OF SEVERELY OBESE ADULTS IN INTERVENTION STUDY**

Subject characteristics by group assignment are presented in Table 12. Gender, age, height, weight, FFM, percent BF,  $VO_{2peak}$ , CV fitness (relative peak oxygen consumption,  $VO_{2peak}$ ), and PA were also not significantly different between groups ( $p>0.05$ ), but BMI ( $p=0.046$ ) and FM ( $p=0.047$ ) were significantly different across groups. Although frequency of Caucasians ranged from 50% in the Diet plus Exercise (DE) group to 87.5% in the Surgery only (S) group, the proportion of African Americans was not significantly different across intervention groups ( $p=0.58$ ). However, analyses to compare participant characteristics across groups were underpowered to detect clinically meaningful differences (i.e. a sample size of 24 achieves 80% power to detect an effect size of 0.64). The majority of participants were middle aged, Caucasian females. By study design, the participants were primarily severely obese with low cardiovascular fitness. All participants self-reported their physical activity level as sedentary. The activity monitors revealed that the average minutes of moderate and vigorous physical activity per week was indicative of a physical active lifestyle defined by the American College of Sports Medicine and American Heart Association (Haskell, 2007).

**Table 12.** Baseline subject characteristics by intervention group (n=24) presented in medians and quartiles unless otherwise stated.

Characteristics	Surgery (n=8)	Surgery + Exercise (n=8)	Diet + Exercise (n=8)
Female (n, %)	8 (100)	7 (87.5)	7 (87.5)
Caucasian (n, %)	7 (87.5)	6 (75.0)	4 (50.0)
Age (yr)	43 (34, 52)	53 (37, 55)	47 (44, 51)
Height (cm)	163.6 (159.3, 171.6)	167.6 (163.7, 169.9)	163.7 (160.4, 171.7)
Weight (kg)	98.8 (90.9, 111.9)	101.1 (94.5, 121.5)	116.7 (111.7, 125.3)
BMI (kg/m <sup>2</sup> )	36.9 (34.8, 38.9)	35.9 (34.1, 44.8)	42.8 (38.5, 44.7)
FFM (kg)	53.4 (43.9, 54.6)	52.4 (46.6, 63.0)	53.0 (50.1, 59.2)
FM (kg)	45.7 (44.2, 53.4)	47.9 (45.1, 53.7)	58.0 (55.5, 60.8)
BF (%)	48.1 (46.1, 51.2)	48.5 (43.7, 51.6)	51.3 (43.6 53.0)
PA (min/wk)	220.0 (188.4, 288.6)	277.5 (147.5, 378.3)	191.4 (87.5, 240.1)
VO <sub>2peak</sub> (l/min)	1.93 (1.49, 2.49)	1.77 (1.54, 2.18)	1.97 (1.74, 2.17)
CV Fitness (VO <sub>2peak</sub> , ml/kgFFM/min)	20.1 (15.1, 22.3)	17.1 (12.3, 19.7)	17.0 (15.7, 18.4)

BMI – body mass index; FFM – fat free mass; FM – fat mass; BF – body fat; PA - physical activity; VO<sub>2peak</sub> – peak aerobic capacity; CV Fitness – cardiovascular fitness

Percent body weight lost and change in physical activity between baseline and post-intervention are presented in Table 13. There was a significant difference in percent body weight lost at the end of the interventions ( $p < 0.01$ ). As a result of the intervention, the S group decreased PA, whereas the SE and DE groups increased PA, but the differences were not statistically significant ( $p = 0.91$ ).

**Table 13.** Changes in body weight and physical activity by intervention group (n=24) from baseline to 6 months. Presented in medians and quartiles.

Characteristics	Surgery (n=8)	Surgery + Exercise (n=8)	Diet + Exercise (n=8)
Body weight lost (%)	-20.5 (-23.2,-18.0)	-23.7 (-27.3,-20.2)	-7.2 (-11.3,-4.5)
PA (min/wk)	-8.6 (-71.7, 193.7)	32.5 (-69.6, 266.1)	36.0 (-73.9, 95.1)

PA - physical activity

#### **4.9 DIFFERENCES IN SKELETAL MUSCLE FATTY ACID OXIDATION CAPACITY AND INTRAMYOCELLULAR LIPID CONTENT ACROSS INTERVENTION GROUPS**

Differences in skeletal muscle oxidative capacity (i.e. percent type 1 muscle fibers and SDH content) and IMCL content within each muscle fiber type and total muscle fibers are presented in Table 14. There was no significant difference across intervention groups in differences in skeletal muscle markers of oxidative capacity or IMCL content in any model ( $p>0.05$ ; Table 14).

**Table 14.** Differences in skeletal muscle oxidative capacity and intramyocellular (IMCL) content across intervention groups. Presented in medians and quartiles.

Outcome Variable	Surgery Only (n=8)	Surgery and Exercise (n=8)	Diet and Exercise (n=8)	Regression Model controlling for Body Weight Lost	Regression Model with All Covariates*
type 1 muscle fiber (%)					
Baseline	51.2 (32.5,62.9)	46.9 (39.1,57.6)	45.1 (40.2,59.1)		
6 months	48.0 (39.4,67.3)	49.4 (29.0,55.8)	48.2 (34.6,58.7)		
Difference	6.9 (-3.1,13.6)	1.3 (-15.8,20.5)	-6.2 (-15.8,20.5)	p=0.93	p=0.75
SDH (AU)					
Baseline	34.6 (23.0,40.6)	34.6 (32.5,38.1)	27.7 (25.0,31.7)		
6 months	32.9 (28.7,38.3)	37.8 (35.5,39.4)	36.1 (30.4,38.4)		
Difference	2.2 (-5.2,2.1)	2.8 (-0.48,3.7)	7.0 (1.7,10.3)	p=0.13	p=0.07
IMCL/type 1 (AU)					
Baseline	5.8 (4.2,7.5)	5.0 (3.0,7.2)	6.0 (4.7,6.7)		
6 months	4.6 (2.8,7.9)	5.1 (3.5,7.5)	6.4 (5.4,6.9)		
Difference	-0.58 (-2.0,0.17)	0.46 (-0.43,1.0)	0.42 (-0.94,1.4)	p=0.46	p=0.25
IMCL/type 2 (AU)					
Baseline	2.8 (1.9,4.7)	2.6 (1.5,3.8)	3.6 (2.2,5.1)		
6 months	2.6 (1.5,3.8)	2.2 (1.7,4.2)	3.6 (3.0,4.7)		
Difference	0.25 (-0.24,0.83)	0.25 (-0.24,0.83)	0.26 (-1.5,0.91)	p=0.13	p=0.48
IMCL/total (AU)					
Baseline	8.6 (6.3,13.1)	7.6 (4.6,10.8)	9.5 (6.9,12.1)		
6 months	6.8 (4.3,10.7)	7.3 (5.1,11.2)	10.3 (8.5,11.9)		
Difference	-2.1 (-3.3,0.37)	1.1 (-0.32,1.5)	0.54 (-1.3,1.8)	p=0.16	p=0.29

\* multivariate analysis controlled for the following covariates: baseline values of age, gender, race, body mass index, cardiovascular (CV) fitness (relative  $\text{VO}_{2\text{peak}}$ ) and PA, and percent of body weight lost. SDH – succinate dehydrogenase; IMCL/type 1 – intramyocellular (IMCL) content within type 1 muscle fibers; IMCL/type 2 – IMCL content within type 2 muscle fibers; IMCL/total – IMCL in total muscle fibers



#### **4.10 CHANGES IN SYSTEMIC FATTY ACID OXIDATION AT REST ACROSS INTERVENTION GROUPS**

Table 15 presents changes in systemic FAO at rest by intervention groups. There were no significant differences across intervention groups for any changes in systemic FAO at rest in either model.

**Table 15.** Changes in systemic fatty acid oxidation (FAO) at rest across intervention groups. Presented in medians and quartiles.

Outcome Variable	<b>Surgery Only (n=8)</b>	<b>Surgery and Exercise (n=8)</b>	<b>Diet and Exercise (n=8)</b>	<b>Regression Model controlling for Body Weight Lost</b>	<b>Regression Model with All Covariates*</b>
RER					
Baseline	0.75 (0.70,0.78)	0.71 (0.70,0.74)	0.78 (0.76,0.80)		
6 months	0.79 (0.74,0.81)	0.75 (0.67,0.80)	0.80 (0.76,0.80)		
Difference	0.03 (0.01,0.06)	0.04 (-0.05,0.12)	0.00 (-0.02,0.02)	p=0.53	p=0.67
Absolute FAO (g/min)					
Baseline	105.9 (97.3,113.0)	125.4 (97.7,131.5)	101.0 (96.0,115.6)		
6 months	80.2 (72.5,90.0)	81.8 (60.5,134.0)	90.2 (81.8,96.4)		
Difference	-23.5 (-39.4,-10.4)	-19.2 (-63.5,75.2)	-20.0 (-25.9,2.5)	p=0.84	p=0.87
Relative FAO (mg/kgFFM/min)					
Baseline	2.1 (1.9,2.2)	2.0 (1.9,2.6)	2.0 (1.7,2.0)		
6 months	1.6 (1.4,2.0)	1.6 (1.3,2.6)	1.6 (1.4,1.8)		
Difference	-0.37 (-0.58,-0.19)	-0.37 (-1.19,0.06)	-0.28 (-0.50,-0.02)	p=0.70	p=0.84
EF (%)					
Baseline	83.9 (72.6,98.3)	97.1 (86.8,100.0)	73.1 (63.5,76.7)		
6 months	68.4 (58.8,86.3)	99.5 (61.7,100.0)	66.1 (63.6,76.7)		
Difference	-11.8 (-22.1,0.56)	0.00 (-36.,10.8)	-1.9 (-10.7,7.1)	p=0.77	p=0.74

\* multivariate analysis controlled for the following covariates: baseline values of age, gender, race, body mass index, cardiovascular (CV) fitness (relative VO<sub>2</sub>peak) and PA, and percent of body weight lost;

<sup>b</sup>different letters denote significant differences within intervention groups (p<0.05).

#### **4.11 CHANGES IN SYSTEMIC FATTY ACID OXIDATION AT ABSOLUTE SUBMAXIMAL WORKLOAD ACROSS INTERVENTION GROUPS**

Changes in systemic FAO at the absolute submaximal workload are presented in Table 16. There were no significant differences across intervention groups for changes in RER ( $p=0.15$ ) and EF ( $p=0.16$ ) during the absolute submaximal workload after controlling for gender, race, percent body fat lost, baseline age, BMI, PA and CV fitness. However, there was a significant difference across intervention groups in changes in RER ( $p<0.01$ ) and EF ( $p=0.01$ ) when only controlling for percent body weight lost. The changes in RER and EF was significantly different in the S and SE groups compared to the DE group ( $p>0.05$ ) with RER and EF having more favorable change in the DE group (Table 16). There was no significant difference between S and SE groups for change in RER ( $p=0.20$ ) or EF ( $p=0.21$ ). There were no significant differences across intervention groups in changes in absolute or relative FAO in either model ( $p>0.05$ ).

**Table 16.** Changes in systemic fatty acid oxidation (FAO) measured during the absolute submaximal workload across intervention groups. Presented in medians and quartiles.

Outcome Variable	Surgery Only (n=8)	Surgery and Exercise (n=8)	Diet and Exercise (n=8)	Regression Model controlling for Body Weight Lost	Regression Model with All Covariates*
RER					
Baseline	0.75 (0.73,0.81)	0.79 (0.75,0.83)	0.83 (0.80,0.84)		
6 months	0.82 (0.81,0.89)	0.85 (0.78,0.86)	0.82 (0.79,0.85)		
Difference	0.08 (0.02,0.12) <sup>a</sup>	0.05 (0.02,0.07) <sup>a</sup>	0.00 (-0.03,0.02) <sup>b</sup>	p<0.05	p=0.15
Absolute FAO (g/min)					
Baseline	332.4 (235.1,420.9)	297.8 (247.2,348.2)	306.0 (256.6,350.0)		
6 months	200.3 (143.5,220.7)	189.4 (149.1,312.2)	257.5 (238.3,310.7)		
Difference	-87.3 (-211.5,-70.7)	-100.6 (-131.7,-63.0)	-31.1 (-79.5,-1.3)	p=0.12	p=0.40
Relative FAO (mg/kgFFM/min)					
Baseline	6.4 (4.5,8.0)	5.5 (4.6,7.4)	5.1 (4.8,6.3)		
6 months	4.0 (2.8,4.9)	4.0 (3.3,5.2)	4.8 (4.1,5.4)		
Difference	-2.0 (-4.3,-0.95)	-1.9 (-2.4,-1.2)	-0.1 (-1.3,-0.5)	p=0.15	p=0.62
EF (%)					
Baseline	82.5 (59.4,92.1)	66.9 (53.8,82.9)	54.7 (48.8,64.3)		
6 months	55.2 (33.5,60.9)	47.0 (40.7,71.8)	54.6 (46.7,66.6)		
Difference	-28.2 (-40.3,-8.4) <sup>a</sup>	-17.9 (-24.6,-9.0) <sup>a</sup>	1.1 (-5.9,9.1) <sup>b</sup>	p<0.05	p=0.16

\* multivariate analysis controlled for the following covariates: baseline values of age, gender, race, body mass index, cardiovascular (CV) fitness (relative VO<sub>2</sub>peak) and PA, and percent of body weight lost;

<sup>a,b</sup>different letters denote significant differences within intervention groups (p<0.05).

#### **4.12 CHANGES IN SYSTEMIC FATTY ACID OXIDATION AT RELATIVE WORKLOAD ACROSS INTERVENTION GROUPS**

Changes in systemic FAO at the relative submaximal workload by intervention groups are presented in Table 17. There was a significant difference across intervention groups for changes in RER when controlling for percent body weight lost only ( $p<0.01$ ) and other proposed covariates ( $p=0.03$ ). When controlling for percent body weight lost only the DE group had more favorable changes in RER compared to the S group (-0.02 vs. 0.07;  $p<0.001$ ) and the SE group (-0.02 vs. 0.04;  $p<0.01$ ). There was no significant difference in change between the S and SE groups ( $p=0.10$ ).

There were no significant differences across intervention groups for changes in absolute FAO when controlling for body weight lost only ( $p=0.06$ ) or the other proposed covariates ( $p=0.10$ ). There was also no significant difference across intervention groups in change in relative FAO when controlling for all proposed covariates ( $p=0.13$ ), but there was a significant difference in change across intervention groups when controlling for body weight lost only ( $p=0.01$ ). In this later model, the DE and SE group had significantly more favorable changes in absolute FAO compared to the S group ( $p<0.01$ ). There was no significant difference in change between the SE and DE groups ( $p=0.31$ ).

There was a significant difference across intervention groups for changes in EF during the relative submaximal exercise bout when controlling for body weight only ( $p<0.01$ ), as well as when controlling for the proposed covariates ( $p=0.03$ ). In both models, the DE group had favorable changes in EF during the relative submaximal workload compared to the S group (6.5 vs. -22.4;  $p=0.00-0.01$ ), but the SE group was not significantly different than the S group (-13.8 vs. -22.4;  $p=0.10-0.13$ ). The DE group also had more favorable changes than the SE group when

controlling for percent body weight lost only ( $p=0.04$ ), but not when controlling for gender, race, baseline age, BMI, PA and CV fitness in addition to percent body weight lost ( $p=0.10$ ).

**Table 17.** Changes in FAO variables during relative submaximal workload across intervention groups. Presented in medians and quartiles.

Outcome Variable	Surgery Only (n=8)	Surgery and Exercise (n=8)	Diet and Exercise (n=8)	Regression Model controlling for Body Weight Lost*	Regression Model with All Covariates
RER					
Baseline	0.81 (0.80,0.86)	0.81 (0.75,0.85)	0.85 (0.81,0.88)		
6 months	0.91 (0.84,0.94)	0.85 (0.78,0.88)	0.83 (0.81,0.83)		
Difference	0.07 (0.04,0.09) <sup>a</sup>	0.04 (0.00,0.04) <sup>a</sup>	-0.02 (-0.05,0.01) <sup>b</sup>	p<0.01	p=0.03
Absolute FAO (mg/min)					
Baseline	335.4 (243.6,453.5)	328.5 (287.5,400.1)	337.1 (214.5,428.5)		
6 months	176.6 (115.8,230.1)	264.5 (191.6,328.9)	295.5 (255.1,324.9)		
Difference	-123.6 (-259.6,-94.1)	-80.0 (-157.1,-41.6)	2.26 (-117.6,62.1)	p=0.06	p=0.10
Relative FAO (mg/kgFFM/min)					
Baseline	7.0 (4.8,8.0)	6.4 (5.3,8.0)	5.9 (4.1,7.6)		
6 months	3.7 (2.2,5.4)	5.2 (4.3,6.2)	5.5 (4.9,6.1)		
Difference	-2.30 (-4.6,-1.5) <sup>a</sup>	-0.67 (-1.4,-0.3) <sup>b</sup>	0.17 (-1.3,0.64) <sup>b</sup>	p=0.01	p=0.13
EF (%)					
Baseline	59.1 (43.2,63.3)	61.1 (46.8,81.9)	46.3 (35.1,59.8)		
6 months	28.4 (16.8,49.0)	46.6 (37.0,70.6)	53.8 (47.0,61.5)		
Difference	-22.4 (-33.6,-12.3) <sup>a</sup>	-13.8 (-15.3,-0.3) <sup>a</sup>	6.5 (-2.1,16.6) <sup>b</sup>	p<0.01	p=0.03

\* multivariate analysis controlled for the following covariates: baseline values of age, gender, race, body mass index, cardiovascular (CV) fitness (relative  $\text{VO}_{2\text{peak}}$ ) and PA, and percent of body weight lost.

<sup>a,b</sup>different letters denote significant differences within intervention groups (p<0.05)

#### **4.13 RELATIONSHIPS BETWEEN CHANGES IN MARKERS OF SKELETAL MUSCLE OXIDATIVE CAPACITY AND INTRAMYOCYELLULAR CONTENT AND CHANGES IN SYSTEMIC FATTY ACID OXIDATION**

None of the changes in markers of skeletal muscle oxidative capacity (i.e. changes in percent type 1 muscle fibers and SDH) and IMCL content were significantly related to changes in systemic FAO at rest or during exercise (i.e. changes in RER, absolute and relative FAO and EF).

#### **4.14 RELATIONSHIPS BETWEEN PHYSICAL ACTIVITY ON SKELETAL MUSCLE AND CHANGES IN SYSTEMIC FATTY ACID OXIDATION**

When controlling for percentage of body weight lost, physical activity (defined either by amount of PA, min/wk, at the 6 month time point or as the change in PA, min/wk, from pre to post intervention) was not significantly related to changes in markers of skeletal muscle fatty acid oxidation and IMCL content or changes in systemic FAO ( $p>0.05$ ).



## **5.0 DISCUSSION**

### **5.1 MARKERS OF SKELETAL MUSCLE OXIDATIVE CAPACITY AND INTRAMYOCELLULAR LIPID CONTENT ACROSS BMI SPECTRUM**

Lower rates of fatty acid oxidation (FAO), both at the level of the whole body and within skeletal muscle tissue, have been reported in obesity and linked to comorbidities such as insulin resistance and type 2 diabetes (Kelley, 1999). This study did not confirm that such perturbations in systemic and skeletal muscle FAO in the postabsorptive, rested state existed in subjects ranging from normal weight to severely obese according to body mass index (BMI) classifications. However, when inducing stress on the metabolic system via moderate intensity exercise, variations in FAO across BMI status were revealed such that greater FAO was associated with higher BMI levels. Submaximal exercise is a good model to examine skeletal muscle FAO since fat represents a major fuel for skeletal muscle during exercise at moderate and low intensity.

In the present study, BMI was not significantly associated with systemic FAO at rest, nor did it explain a significant amount of the variance in FAO. These findings are consistent with previous reports that total FAO at rest did not differ between lean and obese (Goodpaster, 2002), and severely obese individuals (Thyfault, 2004) when FAO was measured by similar methodology utilizing indirect calorimetry. However, our results are not in accord with reported associations between whole body RER and prospective weight gain (Zurlo, 1990). The current

data are also not supported by prior studies that observed lower rates of FAO in obese adults, particularly those with severe obesity, when measuring FAO *in vivo* by indirect calorimetry combined with tracer methodology in limb balance studies (Colberg, 1995; Kelley, 1999), as well as *ex vivo* in muscle homogenates (Kim, 2000) and intact muscle strips (Hulver, 2003). These findings that suggest a defect in FAO at the level of the skeletal muscle appear to be in conflict with the findings of the current study as well as others who did not find a difference in FAO among BMI groups (Goodpaster, 2002; Thyfault, 2004). Yet when considering that skeletal muscle contributes to 17 to 24% of whole body FAO at rest (van Hall, 2002), it is likely that impairments in resting FAO at the level of the skeletal muscle are not reflected at the level of the whole body when measured by indirect calorimetry. Therefore, skeletal muscle tissue and its metabolic properties may be the key factor relating to the ability to oxidize fatty acids for fuel during resting conditions.

Markers of capacity for FAO, including muscle fiber type and as succinate dehydrogenase (SDH) enzyme content assessed with histochemical staining within skeletal muscle biopsies, were not associated with obesity in the current study. These results are in apparent contrast with previous studies suggesting that obese individuals present with a skeletal muscle phenotype characteristic of a limited the capacity for FAO. One longitudinal analysis found that a lower percentage of type 1, highly oxidative, slow-twitch muscle fibers, predicted an increase in body fat over a 19-year period (Karjalainen, 2006). Additionally, a lower proportion of type 1 muscle fibers in a variety of muscle groups has been reported to be associated with obesity defined by either BMI (Hickey, 1995), percent body fat (Lillioja, 1987; Wade, 1990), or trunk fat (Helge, 1999). Other markers of skeletal muscle oxidative capacity, e.g. activity of oxidative enzymes such as SDH, have also been observed to be lower in obese individuals (He,

2001; Kelley, 1999; Kim, 2000; Simoneau, 1999).

There are several possible explanations that may explain the discrepancy between these previous studies and the current results. Although a large variation can exist in human skeletal muscle fiber type proportion and enzyme activity (Simoneau and Bouchard, 1989), the values for percent type 1 muscle fibers reported for this study are similar to previous results of other studies using similar methodology (Hickey, 1995; Tanner, 2002). The lack of association suggests that any variance in the markers related to skeletal muscle's capacity to oxidize fatty acids was not due to adiposity as estimated by BMI. It is possible that the significant relationship between muscle fiber type distribution and BMI previously found could be specific to the gender of the study groups. Lillioja *et al* (1987), Wade *et al* (1990) and Helge *et al* (1999) all conducted their studies in male participants whereas the current study primarily contained females. Previous studies have observed a greater percent of type 1 fibers in females compared to men (Simoneau & Bouchard, 1989). Thus, the current study may not be in direct conflict of previous studies; rather, the results of this study may have identified that the female gender is a protective characteristic that obscures the association between proportion of skeletal muscle fiber type and the onset of obesity. Additional studies should be conducted to examine gender differences among the severely obese.

The potential inference of a gender effect does not explain the conflicting evidence regarding the lack of a significant association between SDH and BMI status. He and colleagues (2001) controlled for sex when comparing SDH within different muscle fiber types from lean and obese participants and did not find a gender effect. Perhaps analyzing SDH by fiber type would have shown a difference in the current study's dataset. It is also possible that the histological determination of SDH may not be adequately sensitive to detect associations

between oxidative capacity in skeletal muscle and obesity, and that other more direct measures of mitochondrial oxidative capacity would have revealed these associations as previously reported (Kelley, 1999; Kim, 2000; Simoneau, 1999)

In addition to skeletal muscle oxidative capacity, skeletal muscle can also be characterized by the storage of lipids within the tissue. Previous studies using immunohistochemical staining of percutaneous muscle biopsy samples observed greater IMCL content in obese compared to normal weight individuals (He, 2001; Goodpaster, 2001; Malenfant, 2001), as well as extremely obese individuals compared to normal weight, overweight and obese individuals (Hulver, 2002). In particular, greater IMCL content in obese appears to be specific to type 1 muscle fibers versus type 2 muscle fibers (He, 2001; Malenfant, 2001). Overall, these studies support the hypothesis that as BMI classification levels increase from normal weight to extremely obese, a possible derangement in fat partitioning at the level of the skeletal muscle exists such that a greater proportion of fat taken up by the muscle is stored as IMCL instead of being oxidized.

In the current study, IMCL content by muscle fiber type and total muscle fibers measured tended to be higher with higher BMI, but the associations were not statistically significant. The heterogeneity of the current study's sample may be a possible explanation for the contradictory evidence found between this study and previous reports. The current study included Caucasians and African Americans whereas none of the previous studies reported race of their sample pool (He, 2001; Hulver, 2002; Goodpaster, 2001; Malenfant, 2001). To date, no study has directly compared if IMCL content differs between these ethnicities. The current study attempted to control for this possible confounding variable by including race (specifically African American ethnicity) as a covariate in the multivariate model used to examine the

association between BMI and IMCL content, and was not found to be a significant predictor of IMCL content.

An additional confounding variable of IMCL content is physical activity and fitness. Goodpaster *et al* (2001) showed IMCL content was greater in endurance-trained adults compared to sedentary but otherwise healthy adults. Previous studies controlled for physical activity level and fitness by recruiting sedentary individuals, but did not directly measure of these variables (He, 2001; Hulver, 2002; Goodpaster, 2001; Malenfant, 2001). This study confirmed a sedentary lifestyle by implementing a graded exercise test to measure cardiovascular fitness. In addition, physical activity monitors provided an objective measure of amount of moderate and vigorous physical activity (PA) per week. Both cardiovascular fitness and PA were then controlled for in the multivariate models examining the relationship between BMI and IMCL content.

It should be noted that IMCL is a measure of all neutral lipids stored within skeletal muscle. Recent studies suggest that lipid intermediates of lipid metabolism, such as diacylglycerol (DAG), fatty acyl-CoA and ceramide, may be more deleterious to metabolic pathways (Adams, 2004; Moro, 2009) than triglycerides per se. Therefore, it is possible that muscle of obese participants in the current study could contain higher levels of these lipotoxic intermediates. This needs to be examined in a future study.

The current study also sought to address if the capacity of obese adults to oxidize fatty acids during physical activity was altered by the severity of obesity. Two previous studies found no difference in systemic RER at rest when lean, matched controls were compared to obese men (Goodpaster, 2002) or extremely obese women (Thyfault, 2004), but did find a lower RER in the obese adults completing 60 min exercise bouts at 50%  $\text{VO}_{2\text{max}}$  compared to the lean adults. In the present study, higher BMI was significantly associated with greater absolute FAO

at the absolute submaximal workloads. BMI explained 46 % of the variance in absolute FAO at the absolute workload. The current study is the first to compare rates of FAO during a submaximal exercise bout at an absolute workload among adults with a wide range of BMI levels from normal weight to severely obese. The results were expected given that those with a higher BMI and lower cardiovascular fitness were working at a higher percentage of  $\text{VO}_{2\text{peak}}$ , thus expending more calories and promoting greater FAO. At the relative submaximal workload, BMI was also significantly associated with absolute FAO, and explained 21% of the variance at the workload relative to 60%  $\text{VO}_{2\text{peak}}$ . However, these results should be interpreted with caution given that expressing FAO relative to FFM, RER or EF, BMI was no longer significantly associated with FAO when controlling for confounding variables at either submaximal workload. The discrepancies may be a result of several factors. First, the degree of adiposity among the current study's lean group was higher than those of lean participants included in previous studies, possibly inducing a phenotype similar to the obese and severely obese groups in this study. Additionally, previous studies have measured differences in plasma metabolites and hormones among BMI classifications, but Goodpaster *et al* (2002) found that these did not relate to FAO during submaximal exercise.

In addition, these findings may be a result of the exercise protocol implemented. Both Goodpaster *et al* (2002) and Thyfault *et al* (2004) measured FAO at a submaximal exercise intensity relative to 50%  $\text{VO}_{2\text{max}}$  whereas the current study measured FAO at a workload relative to 60%  $\text{VO}_{2\text{peak}}$ . Previous studies have found that the exercise intensity that induces greater carbohydrate utilization and less fat utilization is between 48 and 53% of  $\text{VO}_{2\text{max}}$  (Brooks 1998; Venables, 2005). Therefore, the exercise intensity implemented for the current studies protocol may have been too high to elicit maximal rates of FAO, thereby allowing the detection of

differences. An additional point of consideration is the duration of the exercise bout. The submaximal exercise bout implemented by participants by Goodpaster *et al* (2002) and Thyfault *et al* (2004) was sustained for 60 min whereas the current study implemented the relative workload for 10 min. The longer duration may be needed to induce greater stress on the metabolic system to observe differences in FAO across the BMI spectrum during this exercise protocol. Lastly, it is also possible that physical fitness is a stronger determinant of FAO, and that we did not observe associations between fitness and FAO because of the low and fairly narrow range of fitness in the current study.

To summarize this aspect of the study, the findings of the current study suggest that obese individuals, including severely obese individuals, are not limited in their ability to oxidize fatty acids at rest or during exercise, regardless if the exercise bout is set at an absolute workload or made relative to aerobic capacity. These findings have important implications for developing optimal weight loss and weight maintenance interventions among obese individuals, including the severely obese adults. Inclusion of physical activity in such interventions may help to compensate for a positive fat balance due to overconsumption and/or lack of energy expenditure.

## **5.2 RELATIONSHIP BETWEEN SKELETAL MUSCLE AND SYSTEMIC FATTY ACID OXIDATION**

A key hypothesis tested in the current study was that the intrinsic oxidative capacity of muscle tissue measured *ex vivo* would be related to rates of FAO assessed *in vivo*. For example, less highly oxidative muscle fibers, i.e. type 1 muscle fibers would diminish the capacity to metabolize fatty acids such that a lower rate of FAO would be observed at the whole body. In the

current study, a higher proportion of type1 muscle fibers had a significant moderate association with higher absolute FAO during the relative submaximal workload. Variation in fiber type distribution was not explained by BMI in this study so it cannot be postulated that this marker of skeletal muscle oxidative capacity was related to greater rates of FAO during the relative submaximal exercise bout in obese.

The other significant relationship revealed was that between higher IMCL content and higher systemic FAO at rest. This association could be due to higher amounts of fat stored within skeletal muscle are the primary source of fat for fuel during postabsorptive, resting conditions (Goodpaster, 2002; Thyfault, 2004). In addition to skeletal muscle, liver and other energy-utilizing organs rely more on fat during the fasting condition, thus driving higher rates of FAO, rather than the converse, i.e. reduced rates of FAO leading to greater fat accumulation. Although this is purely speculative, the literature is inconsistent as to whether impaired rates of FAO within tissue are actually causing fat accumulation. Further investigation is required.

### **5.3 CHANGES IN FATTY ACID OXIDATION ACROSS WEIGHT LOSS INTERVENTION GROUPS**

The current study employed weight loss interventions in a severely obese cohort to assess the added effect of exercise to weight loss in severely obese adults on changes in skeletal muscle oxidative capacity and intramyocellular lipid content, as well as systemic FAO at rest and during exercise. The weight loss interventions implemented included: 1) bariatric surgery alone (S); 2) bariatric surgery with exercise (SE); and 3) dietary caloric restriction with exercise (DE). There were no across intervention group effects on changes in skeletal muscle or systemic,



postabsorptive resting FAO. However, when inducing stress on the metabolic system via exercise, differences in changes in FAO across intervention groups were revealed with the DE group having more favorable changes in systemic FAO during the absolute workload and relative submaximal workloads compared to the S and SE groups. Although changes in systemic FAO during the submaximal exercise conditions were more favorable in the SE group compared to the S group, the changes were not statistically different.

The majority of studies inducing weight loss with caloric restriction or bariatric surgery alone report no beneficial effect on postabsorptive FAO at the level of the skeletal muscle. After 20% body weight lost, previous obese individuals had no change in fiber type distribution. Caloric restriction alone has shown to have inconsistent effects on oxidative enzyme capacity; studies report no change in carnitine palmitoyltransferase, a decrease in cytochrome c oxidase (Kelley, 1999) and an increase in SDH (Kern, 1999). Even more substantial weight loss of about 50 kg of body weight with bariatric surgery alone did not change gene expression of oxidative enzymes, nor improve skeletal muscle FAO as measured by tracer methodology in muscle homogenates (Berggren, 2008). However, the addition of a 10 d endurance training program (60 min/d at ~70% of  $\text{VO}_{2\text{peak}}$ ) in previously extreme obese adults resulted in positive improvements in skeletal muscle FAO and oxidative enzyme content (Berggren, 2008). In the current study, the addition of exercise to weight loss interventions resulted in no differences in changes in the content of the oxidative enzyme SDH, nor were there differences across intervention groups for change in percentage of type 1 fibers. The current study suggests that there is no difference in changes in markers of skeletal muscle FAO or IMCL content among the weight loss interventions. The results suggest that the groups responded similarly to the interventions. However, such conclusions should be interpreted with caution; this study utilized

methods that examined markers of skeletal muscle FAO capacity. Future research needs utilize more direct measures of these parameters in the postabsorptive state such as those measures of mitochondria content and functional capacity of mitochondria employed by Menshikova *et al* (2005) and Bruce *et al* (2005). Use of muscle cultures also allows for examination of metabolic pathways, especially the activity of these pathways within the mitochondria.

The study also examined changes in neutral lipid content across the three intervention groups, and found no difference in changes in IMCL content across intervention groups. Although this might suggest that the method of weight loss does not affect IMCL content differently, it should also be noted that previous studies have not observed changes in IMCL content after weight loss with or without exercise. He *et al* (2004) who found no change in IMCL in response to a ~10% weight loss induced by caloric restriction and physical activity in previously sedentary overweight and obese adults. IMCL measured by proton magnetic resonance spectroscopy was also not different in overweight participants after a six-month weight loss intervention that employed caloric restriction with or without exercise or very low calorie diet alone (Larson-Meyer, 2006).

Without differences in change in skeletal muscle FAO capacity and IMCL content across groups, it was no surprise that this study also did not find a difference among intervention groups in postabsorptive, resting FAO at the level of the whole body. These results were not expected though. With a mere weight loss of about 2 kg of body weight over 6 months in obese women, the addition of exercise (progressed to 45-60 min, 3 d/wk at 65-70% of heart rate reserve for 6 months) to caloric restriction blunted the decline in resting, systemic FAO observed in individuals losing weight by caloric restriction alone (Nicklas, 1997). Perhaps when more clinically significant weight loss is induced such as that observed in the current study's

participants, even the addition of exercise can not provide more favorable outcomes in systemic FAO at rest.

Although no differences in skeletal muscle or systemic FAO at rest were observed in the postabsorptive state, differences in systemic FAO during bouts of submaximal exercise were observed between groups. At the absolute and relative submaximal workload, the DE group had more favorable changes in systemic FAO expressed as RER and EF when compared to the S and SE groups, such that FAO increased among the DE group, but decreased in the S and SE group in both the absolute and relative submaximal workloads. There were no significant differences in changes in systemic FAO expressed as RER or EF during either submaximal workload between the S and SE groups. During the relative submaximal workload, an additional difference across groups was found when FAO was expressed relative to FFM; both DE and SE groups had more favorable changes than the S group.

Without an additional intervention group that induced weight loss by caloric restriction alone, it is difficult to suggest that the favorable changes observed by the DE group was due to the addition of exercise training, especially considering that the SE group did not have more favorable changes in FAO during submaximal exercise compared to the S group. In addition, when examining PA in the total sample, PA (defined as both the amount of PA completed at 6 months and change in PA from baseline) did not relate to changes in systemic FAO during exercise. Since percent body weight lost was controlled for in the multivariate model, it is unlikely that the changes in systemic FAO during submaximal exercise was due to total amount of weight lost. Thus, these results suggest that the favorable outcomes in FAO during submaximal exercise observed in the DE group must be due to some other positive adaptation in FAO that was not measured.

Other factors that could affect changes in systemic FAO at rest or during exercise include release and availability of FFA in tissue and plasma. FFA release from tissue stores may be affected differently by the weight loss intervention. Nicklas *et al* (1997) found that the addition of exercise to a calorie-restricted diet prevented the decrease in FAO that corresponded to a decrease in lipolysis in the diet only group. The current study's use of indirect calorimetry to measure FAO only provides systemic rates of total FFA oxidized, and does not localize the source of the fatty acid oxidized from plasma or nonplasma sources. Future investigation is needed to determine if the capacity to utilize different sources of fatty acids as a fuel source is impacted by weight loss with or without the inclusion of physical activity.

It is important to identify additional limitations of this study that warrants caution when interpreting the results. First, the insufficient numbers of subjects in the diet only group does not allow us to determine if weight loss-induced decreases in systemic FAO during submaximal exercise are specific to weight loss induced by bariatric surgery. In addition, the participants who underwent bariatric surgery were measured postoperatively; this study can not determine how the initial weight lost after surgery might have affected examination of changes in systemic and skeletal muscle FAO at rest and during exercise. Another limitation was that there was no period of weight stabilization achieved before assessment of FAO after the 6-month weight loss interventions were implemented. It would also be of interest to examine if FAO measured at the 6-month time point in the previously severe obese adults compared to FAO of healthy lean controls to compare capacity for FAO.

In summary, weight loss by bariatric surgery alone or in conjunction with exercise, as well as calorie restriction by diet and physical activity, leads to similar changes in markers of skeletal muscle FAO capacity, IMCL content and systemic FAO at rest. Favorable improvements

in FAO are observed when stressing the metabolic system via submaximal exercise when weight loss is induced by calorie restriction by diet with physical activity, but not when weight loss is induced by bariatric surgery with out without exercise. The findings of this study suggested that the addition of a physical activity regimen to weight loss induced by bariatric surgery might prevent the decrement in systemic FAO that occurs with weight loss induced by bariatric surgery alone, but the overall changes in skeletal muscle and systemic FAO were not found to be significantly different between these two groups. It is also important to consider that if changes in markers of skeletal muscle oxidative capacity and IMCL are not different among weight loss interventions, then skeletal muscle metabolism may not be a causal factor in whole body FAO. Further studies are needed to determine whether the addition of physical activity to a weight loss program attenuates or prevents the decrease in energy expenditures during rest and physical activity that in turn may be related to longer term weight loss or maintenance of weight loss.

Based on the current study it appears that there is no relationship between markers of skeletal muscle FAO, IMCL content and FAO in the postabsorptive resting condition and BMI that includes classifications ranging from normal weight to severely obese adults. Further examination with stable isotope tracers and FAO assays using muscle tissue samples need to confirm these findings. Additionally, the current study did not have enough men in the groups to examine how gender influences FAO at rest and during exercise before and after weight loss interventions. Along with gender comparison, future studies should examine how race also impacts these measures.

Another future study that should be performed is to examine FAO at the relative intensity that elicits maximal FAO and how these might differ across the BMI spectrum. A weight loss

intervention should then be implemented using this workload as the exercise prescription to examine how this results in changes in fat metabolism in severely obese.

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